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July 1, 1975 through June 30, 1976

Part II-A

DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

United States. NATIONAL CANCER INSTITUTE

ANNUAL REPORT of program activities

July 1, 1975 through June 30, 1976

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SUMMARY REPORT
OF THE
DIRECTOR

DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE

July 1, 1975, Through June 30, 1976

INTRODUCTION

The Division of Cancer Biology and Diagnosis was established in 1972 to plan and direct general laboratory and clinical research in diagnosis and biology of cancer. In addition to the intramural program, the Division coordinates the activities of the Breast Cancer Task Force and the Lung Cancer Screening Program and manages the collaborative programs in immunology as well as in diagnosis and detection. A close coordination is maintained with all of the other divisions of NCI in areas of common interest.

The intramural research program consists of three broad areas: cancer biology, immunology, and clinical research. A group of laboratories performing basic research in cancer biology includes the Laboratories of Biochemistry, Pathophysiology, Molecular Biology, and Theoretical Biology. Three units: Immunology Branch and the Laboratories of Immunodiagnosis and of Cell Biology, together with a sizeable contract program, are assigned to the Associate Director for Immunology. Finally, three clinical branches work in the fields of dermatology, metabolism, and pathology. A fourth organizational element, under the Associate Director for Program Planning, manages three major contract programs: Cancer Diagnosis, Breast Cancer Task Force, and Cytology Automation.

Organizationally, the Division underwent few changes last year. Due to the retirement of Dr. Heston, the Laboratory of Biology was abolished and the personnel transferred to the Laboratory of Molecular Biology.

The Laboratory of Physiology, after several years of existence without a chief, was united with the Pathophysiology Laboratory, resulting in better coordination of the program.

As noted in the introduction, four laboratories are involved in basic cancer biology research: Biochemistry, Molecular Biology, Pathophysiology, and Theoretical Biology. Overview of this effort is summarized here.

The Laboratory of Biochemistry is directed by Dr. R. F. Goldberger. After a year of organizational growth, it has developed into six sections. The major effort of the Biochemistry of Gene Expression Section was in trying to define the nature of cellular controls over steroid-evoked responses, using somatic cell hybrids. Preliminary results indicate that current models for the mechanism of steroid action may not be satisfactory. In the area of purification of the glucocorticoid receptors, two forms were isolated chromatographically. They are being examined to determine whether their DNA/chromatin binding activities are similar to those of the progesterone receptors. In studies on the control over steroid-induced events, "cybrids" between enucleated L cells and whole T3C1-2 erythroleukemic cells showed a dramatic reduction in induction of hemoglobin by dimethylsulfoxide. Finally, clinical studies have been carried out on steroid receptors in biopsy specimens.

The Biosynthesis Section is concerned with the relationship between cell function and organization at the molecular level. Comparison of submicrosomal localization of prolyl and lysyl hydroxylases showed that 90% of the prolyl hydroxylase was intracisternal, but only half of the lysyl hydroxylase. Study of mouse myeloma DNA polymerizing enzymes led to the separation of three enzymatically active species using DEAE-cellulose chromatography. All three had similar sizes and catalytic properties. In other projects, cellular interactions in the immune response were studied, structures of macromolecules were examined with electron microscope, and the in vivo transcription of covalently-linked cellular and SV40 viral DNA was investigated.

In the Cell Physiology and Oncogenesis Section the major objective is to elucidate the mechanisms of so-called "spontaneous," virus-induced, and chemical carcinogen-induced malignant transformation in cultured cells. Research toward this objective includes studies on effects of benzo (A) pyrene on epithelial cells in tissue culture, correlation between cell shape and tumorigenicity, and between surface topography and neoplastic transformation, effects of light and oxygen on karyotypic stability and cell growth in tissue culture, diagnosis of neoplastic transformation, and the effects of BCG-activated macrophages on cells in tissure culture.

The Cellular Regulation Section studies regulation of gene expression in eukaryotes at the molecular level. The central problem under investigation is the mechanism by which estrogen induces the synthesis of egg yolk phosphoprotein in rooster liver, with special emphasis on the molecules involved in the interactions of hormone, hormone receptor, RNA polymerase, and chromatin that result in the synthesis of transcripts of the phosphoprotein gene.

The Nucleic Acid Enzymology Section is concerned with detection and study of enzymes involved in nucleic acid synthesis and degradation, while the Protein

Chemistry Section emphasizes the physical, chemical, and biochemical aspects of proteins and subcellular particulates involved in the synthetic, control, and differentiation systems of organisms. Problems currently under study include transfer of isolated chromosomes to mammalian cells, granulocyte development, fractionation of cells involved in cellular immune reactions, and techniques for fractionation of proteins.

The Laboratory of Molecular Biology (Dr. I. Pastan, Chief) has been expanded by creation of a new Section on Gene Regulation, which became staffed by the personnel from the Laboratory of Biology. The research effort of the laboratories can be divided into four broad areas:

1. The biochemical basis of malignant transformation with particular emphasis on the role of cyclic AMP and cell surface protein (CSP) in this process. When propagated in tissue culture, malignant cells can be distinguished from normal cells by their altered morphology, decreased adhesiveness, altered motility, increased agglutinability by plant lectins, and their ability to grow in the absence of certain serum growth factors. A major effort has been devoted toward understanding the biochemical basis of these differences.

2. Regulation of gene activity with particular emphasis on the identification of molecules that activate genes and the determination of the structure of the regulatory regions of genes. Studies were continued on the primary structure of cyclic AMP receptor protein of E. coli, this protein being very difficult to sequence because of the inability to obtain fragments near the amino terminus of the molecule. Determination of the sequence of the operator-promotor region of the galactose operon led to identification of that portion of the sequence which binds CRP. Study of the mechanism of action of termination factor rho showed that the intrinsic ATPase activity of rho is required to carry out rho-mediated termination of transcription. Study of the primary nucleic acid structure at termination points of different genes showed that rho factor-enhanced termination results in a definite structural change in the nucleotide sequence with which an RNA molecule terminates.

3. Investigation of how the thyroid cell forms, stores, and releases its hormone and the elucidation of factors that control its growth, differentiation, and transformation into a malignant cell showed that the thyroid cell can phagocytize red blood cells and that pseudopod formation may be induced by the presence of particulate objects such as red cells or pseudopods on adjacent cells.

4. Investigations on mammary tumor virus and other oncornaviruses and elucidation of how these viruses are synthesized and how they change normal cells into cancer cells.

During the past year, Dr. P. Gullino reorganized the Laboratory of Pathophysiology, adsorbing personnel from the abolished Physiology Unit and relocating to new quarters. The research program was carried out in several directions. Studies on the regression process in mammary tumors

after hormonal deprivation of the host showed that as the host is deprived of hormones, single cells in the solid tumor start regressing. The number of regressing cells increases with time and is not determined by systemic metabolic alterations or by an immunological type of tissue rejection. Regression occurs as a cell-centered event with autodigestion as the most prominent feature. Within 24 hours from hormone withdrawal the cytosol proteins become more susceptible to lytic enzymes. In another project, reduction in G6PD activity was observed during regression of DMBA and MTW9 tumors, this reduction being due to the disappearance of microsomal G6PD enzyme. Attempts to develop an *in vitro* system for mimicking the regression of solid tumors continued, but a working model has not yet been produced.

In studying the role of poly(ADP-ribose) in cell cycle regulations, it was previously shown that nuclei of eukaryotic cells polymerized the ADP-ribose moiety of NAD through a 1'-2'-ribose-ribose linkage to form poly(ADP-ribose). Now it has been found that in human cell lines poly(ADP-ribose) electrophoresed with H1 histone as in rodents. A radioimmunoassay was developed for quantitation of poly(ADP-ribose). Poly(ADP-ribose) polymerization was found to decrease with the onset of tumor regression and increase again with tumor regrowth; the same was observed for MCF7 human mammary carcinoma cells *in vitro*.

Another area of interest concerns the behavior of ribosomes and mRNA in resting and growing human lymphocytes. It appears that newly formed ribosomes are utilized 10-15 times in protein synthesis and then become inactivated to the free ribosome form. When lymphocytes are stimulated to grow, free ribosomes dissociate and enter protein synthesis. The activation of free ribosomes does not require new synthesis of protein, but of mRNA.

Investigation of the role of extranuclear DNA and nucleotide metabolism in tissues led to the search for differences between nuclear and cytoplasmic DNA, revealing that DNA was present in melanin granules of S-91 melanoma. Most of mitochondrial DNA renatured very rapidly as a single component, and 65% of microsomal DNA renatured more slowly, still as a single component; both can be considered distinctive types, rich in repeated sequences. In contrast, nuclear DNA renatured slowly, with no well-defined components. Methylcytosine was found in all three sources and could not be used for differentiating these types.

The remaining project areas cover the effects of ionizing and UV radiation on tissue components, nucleic acids, and proteins, and the consequences of tissue destruction by radiation on the host; and, finally, the physiological and behavioral control of food and water intake in relation to cancer cachexia. Extensive analysis of the normal control of feeding has demonstrated that this is a highly redundant system; however, as the tumor develops, a successive deletion of different components of the feeding control mechanism occurs until a catastrophic collapse ensues with cachectic hypophagia.

The activities of the Laboratory of Theoretical Biology (Dr. M. Berman, Chief) fall into several areas: the biophysics of membranes, immunology, molecular configurations, kinetics of metabolic systems, and mathematical and computational methodology and theory related to modeling.

The work on membranes is mostly related to lipid bilayer systems. The study of antibody-dependent lymphocyte-mediated cytotoxicity has been extended to use of the lipid antigen DNP-caproyl phosphatidyl ethanolamine. The lymphocyte-induced conductance changes are found to be highly sensitive to antigen concentration. All lymphocytes in a given incubation incorporate dye from vesicles so that the use of the fluorescence-activated cell sorter has permitted investigation of a variety of issues related to the phenomenology and mechanism of interaction. A relationship has been found between ionophoric activity in bilayers and mitogenic activity in mouse B-lymphocytes in the case of four high molecular weight proteins.

The development of mathematical models for the interpretation of the dynamics of immune response systems was initiated. The binding of multifunctional ligands to a surface with a dense homogeneous array of sites was modeled, and the results applied to the analysis of the phage inhibition assay. For immunoglobulin G the model consists of a two-step process: a bimolecular reaction followed by intramolecular bonding. It was possible to estimate the forward rate constant for the second step from available data. A simple pilot model was also proposed for the interaction of tumor cells with killer lymphocytes. A careful theoretical investigation of this model showed that despite its simplicity it can account for a number of interesting features observed experimentally. For example, the behavior is robust and includes the possibility of tumor-host coexistence at sufficiently low tumor levels.

The modeling of metabolic systems, especially endocrine systems, is continuing. The model previously developed for glucose-insulin regulation in normals has been applied to obese and diabetic individuals, and for each subject a pattern of metabolism has been established which shows sensitivities of glucose production, glucose utilization and insulin secretion. This model is now being further developed to identify primary sites of defect that may explain the observed kinetic patterns in each subject. New features, extensions, and refinements have also been added to the lipoprotein model, especially with regard to the apoproteins, triglycerides, and cholesterol. Modeling of cholesterol metabolism has been initiated. Cholesterol provides a tool through which the lipoprotein particles may be viewed. Studies are also in progress on HDL apoproteins A-I and A-II which show that the two apoproteins, though degraded together, are not synthesized in a fixed ratio to each other. An interesting seesaw degradation process has been observed between vascular and extravascular degradation sites. Studies from endogenously labeled lipoproteins have validated models previously developed and added new information on synthesis and the recycling of hydrolysis products.

Other areas of interest include transport mechanisms between fetus, mother, and amniotic fluid, concentrating on the transport of water, creatinine, and hormone metabolites; kinetic theories dealing with chemical reactions based on stoichiometry oriented approach have been worked out; and, finally, the theories of stability, oscillations, and pattern formations in biological systems have been applied to regenerative processes and to tumor immune responses.

Tumor immunology research comprises the program of four laboratories in the Division of Cancer Biology and Diagnosis. The Immunology and Immunodiagnosis Branches and the Laboratory of Cell Biology are organized under the Associate Director for Immunology. Although the Metabolism Branch is not organizationally connected with this program, its research is summarized in this section because of its content. Tumor Immunology Contract Program is reported in the Contract section.

The Immunology Branch (Dr. W. Terry, Chief) carries out basic immunobiology investigations with emphasis on the structure and function of cell membranes, cellular immunity, genetics of the immune response, and transplantation. The functions of cells initiated and modulated by interactions between cell surface receptors and a variety of ligands. Recent studies have shown that Fc receptors, which can bind antigen-antibody complexes or aggregated IgG, are present on murine B cells, a subpopulation of murine T cells and macrophages. The Fc receptors of B cells and T cells are associated with cell surface Ia antigens. Fc receptors may play a key role in immunoregulation, since it has been shown that mitogenic stimulation of cells bearing Fc receptors can be inhibited by immobilized antigen-antibody complexes.

Studies have been performed on the mechanisms by which lymphocytes kill target cells using lipid bilayer membranes as a model for the target cell membranes. When these membranes contain DNP groups, and antibodies for DNP are allowed to interact with them, subsequent addition of non-immune human lymphocytes causes a conductance increase across the antibody-coated lipid bilayers, suggesting that an increase in ionic permeability may be the basic mechanism responsible for target cell death. Previous studies have shown that attacking cells undergo a marked shape change when they interact with target cells. Lymphocytes that interact with antibody-coated plastic surfaces undergo identical morphologic changes. These changes and the capacity of these cells to kill in the antibody-mediated system can be inhibited by drugs affecting energy metabolism, microtubules, microfilaments, and intracellular cAMP levels.

Investigation of cellular immunity showed that the effect of adherent cells on tumors *in vitro* ranged from inhibition of tumor proliferation to profound stimulation. Adherent cells from BCG-treated mice were less stimulatory and showed defective phagocytosis. This appeared to be due to increased numbers of adherent, but nonphagocytic, cells. Techniques were devised for the isolation of these cells.

An attempt was made to define the defect in the lymphoid system of AKR mice which predisposes to the high spontaneous incidence of thymic lymphoma and lymphocytic leukemia. The spleens and lymph nodes of leukemic animals contain an increased proportion of theta positive lymphocytes; normal or preleukemic mice do not show this. Analyses of mitogen responsiveness of lymphoid organs in leukemic AKR mice show that spleen cells have decreased PHA and Con A responsiveness, but that there is a more severe depression in

Con A responsiveness causing an increase in the ratio of PHA to Con A mitogenesis. By contrast, the thymoma cells show a marked absolute increase in PHA responsiveness. The changes in mitogen responsiveness of lymphocytes from leukemic AKR mice may reflect differential susceptibility of subpopulations of cells to infection by murine leukemia viruses. Tumors arising in aged AKR mice that are thymectomized at the age of one month show cell surface characteristics of both T and B cells. When passaged into young AKR mice, the cells express only T cell characteristics, but then differentiate and express both T and B cell markers.

The demonstration of genetic control of immune responses has opened an important pathway in gaining an understanding of the biology of the immune system. A variety of studies are being carried out in an attempt to determine the mechanisms of the genetic control as well as to determine the biologic significance of this type of control. Specifically, an in vitro systems in which F1 responding lymphocytes generate cytotoxic effector cells against spleen cells of $H\text{-}2^b$ parents but not against $H\text{-}2^a$ parents has been developed. This system provides a model for elucidating the recognition and effector phases of hybrid resistance to parental hemopoietic grafts and may be useful in developing matching procedures for clinical bone marrow transplantation.

In an attempt to determine whether Ir genes control the immunoglobulin variable regions that are produced in "genetically controlled" antibody responses, it has been shown that idiotype expression on mouse antinuclease antibodies is linked to heavy chain allotype markers and not to the immune response gene locus.

Studies of the structure and function of the constant portions of immunoglobulins are in progress. The interaction between the constant portion of rabbit antibodies and complement components is being analyzed. In addition, divalent affinity labeled antigens have been used to create antigen-antibody complexes of small defined size and the ability of these complexes to bind to cell surface Fc receptors is being investigated.

A protocol comparing chemotherapy and two different forms of immunotherapy in patients with stage I and stage II malignant melanoma has been initiated.

The research activities of the Laboratory of Immunodiagnosis (Dr. R. Herberman, Chief) can be divided into three areas: cell-mediated immunity studies in animal tumor systems and in man, and immunochemical studies of tumor associated antigens. The main emphasis in each of these areas is on the application of the research information to the diagnosis of cancer and monitoring of disease.

An apparent paradox in the MSV tumor system and other syngeneic systems has been the short-lived cytotoxic cellular immune response, in contrast to the long lasting in vivo resistance to tumor challenge. It was demonstrated that challenge with tumor cells carrying the appropriate antigens resulted in rapid appearance of a secondary cytotoxic response. The ability to detect cytotoxic reactivity was a function of the length of time between primary and

secondary challenge, the site at which the animals were challenged, the site from which effector cells were harvested, the length of time between the secondary challenge and the harvest of the effector cells, and the size of the challenge inoculum. Each of these variables acted independently to influence the kinetics of the secondary cytotoxic response.

The most important issue related to the natural cytotoxicity is its possible in vivo role in resistance against tumor growth. In a series of experiments, several mouse tumors have been found to grow less well in nude mice than in normal mice, all of them being very susceptible to natural cytotoxicity. X-irradiation of nude mice had no deleterious effect on resistance of nude mice to tumor growth, whereas treatment with cyclophosphamide caused enhanced tumor growth.

Major improvements have been made in the ability to detect specific lymphoproliferative responses of immune rats to (C58NT)D tumor cells. Tumor-bearing rats had increased levels of suppressor cells in their spleens, but in contrast to mice, both normal and immune rats had considerable suppressor activity. The suppressor cells could be removed by passage over rayon adherence columns or by removal of phagocytic cells. Depletion of T cells with a specific anti-T cell serum plus complement did not affect the suppressor activity. Therefore, the suppressor cells appear to be macrophages.

The suppressor macrophages have also been found to have strong inhibitory effects on the proliferation of many tumor cells in vitro. In both the mouse and the rat, the growth inhibitory cells appear to be the same as the suppressor cells. The growth inhibition assay has provided a rapid and simple assay for the levels of suppressor cell activity in individual animals.

A study was initiated to evaluate the ability of host cells within primary MSV-induced tumors to function as effector cells in vitro. T lymphocytes have been identified in the tumors by their surface markers and by their functional reactivity. Macrophages were also found within the MSV tumors as determined by phagocytosis, the presence of an Fc receptor on their surface, and by their ability to inhibit the proliferation of lymphoma cells in a growth inhibition assay.

Experiments were performed to relate the in vitro reactivities of lymphocytes with ability to transfer resistance against tumor challenge. Systemic administration of lymphocytes from immune rats could protect against subsequent challenge. Protection was strongest with cells from rats 30-50 days after immunization. Cells taken at 10 days after immunization had only weak protective effects. The adoptive transfer in this tumor system seems to be mediated by memory cells which circulate and migrate to the site of tumor challenge. The ability to transfer resistance was completely dependent on T cells; macrophages or other adherent cells were not required for adoptive protection.

Because of the importance of natural reactivity in various cytotoxic assays, the specificity of these reactions were reevaluated, studying in detail the characteristics of the effector cells. In testing directly normal

peripheral blood lymphocytes against a variety of tumor cell lines, it was confirmed that the myeloid line K-562 was a particularly good target yielding reproducible results.

The inhibition studies with antigen-antibody complexes strongly suggested that the effector cells in the K-562 system bear an Fc receptor and are, at least in this respect, similar to the K cells mediating ADCC. In testing this hypothesis, it was found that Fc receptor on the killer cell is not a K cell, nor does it play any role in the binding to the target cell.

Considerable emphasis has been placed on evaluation of cellular immune competence in cancer patients. Studies have been performed on rosette formation of human peripheral blood lymphocytes with sheep erythrocytes (E-rosettes). With performance of the assay at 29 C, only 50-55% of normal lymphocytes formed E rosettes, compared to 75-80% at the more optimal temperatures of 4 C. The T cells which form rosettes at 29 C were found to be a separable subpopulation of cells with different functions than those which only form rosettes at lower temperatures. It was possible, however, to change the relative proportions of these cells by incubation in vitro with human thymosin.

In related developments, cell-mediated immunity of cancer patients to tumor associated antigens was studied, measuring delayed hypersensitivity reactions of patients to purified soluble fractions of tumor cell extracts. A series of tests was performed in evaluating the LMI reactivity of breast cancer patients to mouse milk containing mammary tumor virus.

Another development has been the modification of the microagarose droplet migration assay for the measurement of LMI. Initial experiments were performed with PPD as antigen, using tuberculin skin test positive and negative donors. This method has already proved useful in testing multiple soluble fractions from tumor cell extracts, and has detected specific reactivity to nanogram concentrations of some fractions.

Extensive studies have been performed on the cryopreservation of effector cells and of target cells in both the clinical and experimental animal systems. This has proved extremely useful in standardizing the assays and minimizing day-to-day variations.

Both gastric and pancreatic carcinoma have been noted to be associated with CEA, AFP, and HCG. A retrospective study to determine the value of simultaneous measurement of all three markers has been set up. Patients with pancreatic cancer have been evaluated, and the use of combined markers demonstrates a clear superiority.

Studies of a human lung tumor associated antigen have continued. With the use of ion exchange and affinity chromatography, considerable purification has been achieved. Efforts are now being made to produce more specific antisera and to develop more quantitative assays, especially radioimmunoassay. The separated lung tumor associated antigens are also being tested for reactivity in leukocyte migration inhibition assays.

The clinical research program of the Metabolism Branch (Dr. T. Waldman, Chief) is directed toward two major goals: definition of host factors that result in a high incidence of neoplasia and determination of the physiological and biochemical effects that a tumor produces on the metabolism of the host.

Sensitive radioimmunoassays for the oncofetal proteins alphafetoprotein (AFP) and human chorionic gonadotropin (hCG-β) were established in previous years and shown to be of value in the diagnosis of certain forms of malignancy. The radioimmunoassays for AFP and hCG were evaluated as adjuncts to the diagnosis of testicular malignancy and in monitoring the effectiveness of therapy of this disorder. The use of markers appears to be of value as an adjunct to the diagnosis, and of even greater value in monitoring the effectiveness of therapy of the testicular tumor. All elevated values of AFP or hCG in patients with germ cell tumors reflect residual tumor. The presence of a positive marker should be taken into consideration in determining the future course of the patient's therapy.

A significant effort has been directed toward defining the nature of cellular differentiation, cellular interaction, and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens and to define the pathological defects in patients with a primary immunodeficiency or with a malignancy of the T- or B-lymphocyte system. The majority of patients with primary immunodeficiency diseases as well as patients with chronic lymphocytic leukemia appear to have a defect in the terminal differentiation of B-lymphocytes into immunoglobulin synthesizing and secreting cells. Patients with thymoma and associated hypogammaglobulinemia had excessive numbers of activated suppressor T-cells which may play a role in the hypogammaglobulinemia observed in these patients.

The nature of the defect in patients with isolated IgA deficiency was investigated using the in vitro technique to study pokeweed mitogen-driven terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. These patients can synthesize IgA but appear to have a defect in IgA secretion. A smaller group of patients with isolated IgA deficiency did not synthesize IgA and had IgA specific suppressor cells that prevent B-cells from maturing into IgA synthesizing and secreting cells.

Studies of the immunoregulatory role of T-cells have been pursued in an experimental animal model of humoral immunodeficiency. In avian species, a central lymphoid organ, the bursa of Fabricius, is the site of initial differentiation of B-lymphocytes. Removal of the bursa results in agammaglobulinemia in the birds that survive, characterized by total deficiency of B-lymphocytes and plasma cells, absence of germinal centers in the lymphoid tissue, and a total absence of serum IgM, IgA, and IgG. Cellular immune function, however, is preserved. T-cells from agammaglobulinemic chickens have profound effects on humoral immune responses when transplanted into normal birds, the recipients developing agammaglobulinemia despite the presence of a bursa of Fabricius. Thus, the immune defect in these T-cell graft recipients closely parallels the defect observed in

many patients with common variable hypogammaglobulinemia. Further studies have shown that suppressor T-cells develop in bursectomized birds after all Ig has disappeared from their circulation and that such suppressor T-cells have specificity for each of the immunoglobulin classes. Thus, when T-cells from bursectomized agammaglobulinemic birds are transplanted into normal recipients, some develop deficiency of all classes of Ig while others develop dysgammaglobulinemia, characterized by loss of some, but not all, classes of Ig. T-cells obtained from these dysgammaglobulinemic birds are then capable of transferring this suppression to other birds but do not suppress Ig classes which were normal in the donor chicken. Thus, animals with selective deficiency of IgG, or IgA, or IgM, as well as birds with deficiency of IgG and IgA, or IgM and IgA, or IgM and IgG have been produced. This Ig class specific suppression also closely parallels various human immune deficiency diseases. Patients with multiple myeloma have a significant reduction in the serum levels of polyclonal immunoglobulins and in the capacity to synthesize antibody in response to antigenic challenge. When the in vitro immunoglobulin biosynthesis test was applied to the peripheral blood lymphocytes from patients with myeloma, they showed profound depression of immunoglobulin synthesis to approximately 10% of the normal mean values. Peripheral blood lymphocytes from the majority of patients with myeloma tested suppressed the polyclonal immunoglobulin synthesis of normal lymphocytes when co-cultured with them.

Studies directed at the pathogenesis of gluten-sensitive enteropathy (GSE) was continued. GSE is a disease of the gastrointestinal tract associated with malabsorption, villous flattening, and increased incidence of neoplasia. In previous studies, 80% of patients with GSE carried the HLA-B8 histocompatibility antigen. In current studies a second lymphocyte antigen associated with GSE and dermatitis herpetiformis was identified. In family studies it was observed that the gene responsible to this antigen is not linked to the HLA-complex. This antigen may be the product of the Ir gene that is postulated to occur in GSE.

Studies of the cells mediating cytotoxicity reactions led to an understanding of both mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC) reactions. Human peripheral blood cells were fractionated yielding polymorphonuclear cell-rich populations; purified lymphocytes; purified B-cell populations; T-cell populations containing Fc receptor-positive, SIg-negative cells; and T-cell populations depleted of Fc-receptor positive, SIg-negative cells. All cell populations were found to contain effector cells in MICC using chromated chicken RBC targets, whereas polymorphonuclear cells, macrophages as well as Fc-receptor positive, S1g negative cells but not B-cells or T-cells could function as effector cells for these targets in ADCC.

Humoral factors playing an important role in immunoregulation have been extensively studied. One of the most striking examples of immunoregulation is found during pregnancy when the histoincompatible fetus and placenta are protected from immunologic attack by the cells and immunoglobulins of the mother. Human chorionic gonadotropic (HCG) contains potent immunosuppressive activity, demonstrable in vitro as a totally

reversible inhibition of lymphocyte proliferative responses to nonspecific mitogens like PHA, to specific antigens like Candida, and to allogeneic cells in the mixed lymphocyte reaction. HCG also inhibits the ability of lymphocytes, polymorphonuclear leukocytes, and monocytes to kill cellular target cells in a variety of assays. Further studies, however, have shown that highly purified homogenous preparations of HCG do not manifest immunosuppressive activity. A small molecular weight compound present in the urine of pregnant women which co-purified with HCG during its commercial preparation is responsible for the immunosuppressive activity. It is now becoming clear that compounds with the properties of an almost ideal immunosuppressant are present during pregnancy but that these materials have not yet been convincingly identified.

The research program of the Laboratory of Cell Biology (Dr. L. Law, Chief) is multidirectional, ranging from investigations of somatic cell genetics to in vitro studies of cell-mediated immunity to immunochemistry of myeloma proteins.

In the area of somatic cell genetics the detection and location of specific neuron antigens expressed by neuron x fibroblast hybrid cells have been studied. Rodent neuron x human fibroblast hybrid cells have been obtained that are losing human chromosomes and these hybrid cells have been processed through a series of steps and reacted with various portions of the freshly dissected embryonic nervous system. Localization has been observed of fluorescent label on individual neurons especially in one case on a group in the dorsal spinal cord. It is anticipated that this will prove a valuable and general new method with which to study specificity in the developing nervous system of mammals.

Mutants for antibiotic resistance are being used to study recombination in mitochondrial DNA. Recombination of these resistance markers will be mapped by making hybrid and cybrid crosses between the closely related subspecies of mice. At the level of the restriction enzyme patterns of Eco Rl and Hin III the standard laboratory inbred strains of mice are indistiguishable from one another. However, several of the subspecies of M. musculus have mtDNA restriction sites easily distinguished by both Eco Rl and Hin III. It is anticipated that the more complex patterns obtainable by digestion with other restriction enzymes such as Hae III coupled with cRNA-DNA hybridization of these fragments will allow for mapping and the definitive demonstration of mitochondrial recombination in mammals.

Studies of the structural basis of antibody specificity involved labeling the antibody combining site and localizing the label within the primary structure of the antibody molecule. The labels are found within the hypervariable sequences in the variable region of the heavy and/or light chains. Using the paired-iodination technique, a tyrosyl residue at position 30 in the light chain has been identified as being in the binding site. This demonstrates that both the first and third hypervariable regions in the light chains may contribute to the binding site structure.

A highly purified preparation of mouse β_2-microglobulin has been ob-

tained from sodium thiocyanate extracts of liver cell membranes. The first 40 residues have been sequenced. The mouse protein differs only at 9 positions from human β_2-microglobulins. The sequence also has homology to the constant regions of mouse γG2a, most closely to the C_H3 region. These data support the conclusion that the mouse protein is the mouse homologue of human β_2-microglobulin. The nature of this protein remains at this moment unknown. The possibility that other specificities, for example, of the Ia type, are indeed carried by this protein are being actively investigated. This situation resembles the allogeneic effector factor, where both Ia and β_2-microglobulin antigenic determinants have been shown to be present.

In the area of tumor specific antigens attempts at purification of SV-40 TSTA provided some comparative information concerning the relationship of H-2 and TSTA. There is a distinct difference in sensitivity to papain of H-2 and the (SV40)TSTA; H-2 elutes in a sharp peak during gel filtration whereas the specific TSTA elutes in more than one peak; after affinity chromatography, H-2 activity is in the bound Con A II fraction whereas specific TSTA activity is in the unbound Con A I fraction. T-antigen, a nuclear antigen also induced by an "early" SV40 virus gene and detectable by CF assays, has been proposed to provide tumor-rejection activity. The highly immunogenic fraction as well as DEAE I and G150 I fractions having tumor rejection activity did not contain measurable T-antigen activity.

Studies were continued of the "eclipse" phase of growth of the mKSA (SV40-induced) neoplasm. BALB/c mice bearing large tumors are unable to reject this syngeneic tumor and T cells from these tumor bearers are not competent; nevertheless, 10 days following amputation of the tumor, the animal becomes immunologically competent and T cells neutralize tumor cells in a specific fashion. A fraction was found in ascites fluid and in serum of tumor-bearing mice that can extend the "eclipse" period in amputated mice and will provide progressive growth of tissue culture-propagated cells that have lost their oncogenic potential. This factor was found also in DBA/2 mice bearing L1210 ascites but not in ascitic fluid of several other ascitic tumors.

The in vitro studies of cell-mediated immunity includes four projects. In one it was shown that induction of in vitro tumor immunity could only be blocked specifically by the antiserum with appropriate antigenic specificity. The primary and secondary cell-mediated cytotoxic responses were detected in invitro and in vivo with very good correlation, making it possible to study the mechanisms of the in vivo induction of tumor immunity. In another project it was established that some humoral factors are produced in hosts which can affect the tumor growth and host immune response. And finally, in an attempt to characterize soluble antigens it was found that various antigens might be responsible for the induction of cell-mediated immunity.

It has been previously established that intracisternal A particles (IAP) have several biochemical characteristics known to be associated with murine oncornaviruses. It has also been shown that IAP can be detected in

the two-cell embryo, suggesting that vertical transmission can occur. However, until now there has been no evidence that IAP can be transmitted horizontally. Through the use of techniques for somatic cell hybridization, it was possible to transfer IAP from enucleated IAP-positive cells (cytoplasts) to IAP-negative cells by fusing them in the presence of inactivated Sendai virus. Experimental results suggested that the genetic information coding for IAP can be transferred in the cytoplasm and thus horizontal transmission can occur under appropriate conditions.

A new genetic marker linked to the IgC_H locus has been identified LEV2,1.1. The strain distribution of this marker is unique thus far, and will provide a valuable new marker that is easy to identify. The idiotype is cross specific and is found on most B2,1 binding myeloma proteins and on antibodies raised to Aerobacter levan. It is probably a natural antibody in most strains of mice. The absence of the marker in the C57BL strain permitted the establishment of linkage to IgC_H by its characteristic distribution in RI strains and congenic strains.

Considerable effort is devoted to the immunochemistry of myeloma proteins: determination of primary and three-dimensional structure of the antibody combing site of a phosphoryl caoline binding myeloma protein, as well as of proteins with other binding specificities; attempts to determine the amino acid sequence of other galactan binding proteins; and comparative structural studies of immunoglobulins which react with levan-containing antigens.

CLINICAL RESEARCH

The report of clinical research includes work on skin cancer and research of the Laboratory of Pathology. The Image Processing Unit is organizationally a part of the Diagnosis Branch, but its work is intramural; therefore a report is included in this group of summaries. The program of the Metabolism Branch was reported under immunology research and the activities of the Diagnosis Branch are included in the Contracts Program.

The Dermatology Branch (Dr. M. Lutzner, Chief) conducts both clinical and basic research, studying the etiology, diagnosis and treatment of malignant diseases involving the skin and the host's response to these diseases.

Interesting results were obtained from the study of Sezary syndrome, a lymphocytic neoplasm of the T-cell type, characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood.

A number of patients with Sezary's Syndrome have been treated by leukapheresis. Patients seem significantly improved following treatment, but often relapsed requiring further leukapheresis.

It has been found that the Sezary cell has significant B-cell helper function; in these individuals B-cells can be induced to divide and form immunoglobulins by Sezary cells. Karyotypes were studied from 11 Sezary patients. Abundant spontaneous division of heteroploid cells, a high percentage of heteroploid cells in stimulated cultures, and clone formation were found to be indicators of a poor prognosis for the patient with the Sezary syndrome.

Work on animal models for genetic human skin diseases has been initiated; a mutant rat has been found to be the first animal model for human skin blistering diseases.

Xeroderma pigmentosum is a rare autosomal recessive dermatologic disease, characterized by pigmentary changes, telangiectasia, atrophy and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients are histologically identical to those found in otherwise normal persons. A defect in repair of UV damage to DNA has been found in XP cells.

Post-UV colony-forming ability (CFA) in XP fibroblasts from each of the six genetic forms of XP was studied. A striking correlation between the degree of reduction in post-UV CFA and the presence and severity of XP-associated neurological abnormalities has been found. These results constitute the first strong evidence for a relationship between the effective level of DNA repair and the severity of a heredo-degenerative condition of the nervous system.

Studies in this lab have confirmed reports of decreased post-X-ray CFA in cells from patients with ataxia telangiectasia, a disease in which patients have an increased rate of cancer, plus degenerative findings in the

immune and nervous systems. Long-term lymphoblast lines from at least one representative of each of the six genetic forms of XP were established. Work to date with several such lines indicates that they retain the same level of diminished DNA repair as the fresh peripheral lymphocytes from which they arose.

Attempts are being made to accomplish in vitro malignant transformation of XP and normal fibroblasts of UV light. If successful, the results of such work when correlated with the knowledge of DNA repair efficiency and UV mutability should yield important new information concerning the interrelationships of DNA repair, mutagenesis and carcinogenesis.

Pemphigus, herpes gestationis, bullous pemphigoid and dermatitis herpetiformis are blistering skin diseases, all of which are strongly associated with so-called autoimmune phenomena. This Branch was mainly concerned with the identification and characterization of serum and in vivo bound antibodies in the latter 3 diseases, centering around the mechanisms of tissue destruction, ultrastructure, and ultrastructural localization of these antibodies.

A combination of in vitro and in vivo controlled methods for the study of cell-mediated immunity in man made it possible to initiate basic immunopharmacology and immunogenetics approaches for the study of a variety of diseases and resulted in several practical diagnostic procedures. Initial studies offer promise of a predictive index for agents affecting the cellular immune apparatus, which is known to be important in host defense against a variety of neoplastic processes.

The melanosome in normal mouse tissue is composed of multiple proteins, many of which are loosely bound and easily extracted. Another protein of lower molecular weight, tightly bound to the granule, probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. Melanosomal proteins from melanoma tissues vary in structure from those of normal tissue. Several of the proteins seem to be missing completely from these granules, which are structurally distinguishable from normal granules. Many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules. This has been found to be the case in human melanoma as well.

Since only tyrosinase is essential for melanin biosynthesis, it is a unique system for the study of enzymatic control mechanisms in normal and malignant tissues. The status of enzymatic control of melanogenesis was examined, showing that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, but not peroxidase, as has been suggested by some workers. It has been found in this study that tyrosinase is under allosteric control and that phospholipids may play a part in the expression of the enzyme's activity.

Although the Laboratory of Pathology (Dr. L. Thomas, Chief) is heavily involved in histopathology and cytopathology services for patients throughout the Clinical Center, it also carries out a broad research program with major

emphasis in viral oncology and other aspects of carcinogenesis. Noteworthy research results include:

Characterization of the morphology, immunoglobulin production and surface membrane receptors on the cells of human malignant lymphomas and leukemias and relating these to similar studies of the normal cells of origin of these lymphomas and leukemias.

Characterization of the pathogenesis of hepatic fibrosis and the development of angiosarcomas in the liver of workers exposed to vinyl chloride.

Demonstration that propidium iodide - fluorescein isothiocyanate staining shows definite promise as a bicolor-fluorescent system for the automation of cytology screening of cervico-vaginal specimens.

Differentiation of neurovirulent from attenuated strains of Type 1 polioviruses by the structural lability of virion polypeptides.

Demonstration that a membrane Fc receptor which develops on the surface of cells infected with HSV is related to synthesis of early viral products. Engagement of this surface membrane receptor by IgG interferes with viral production. These observations may be important for elucidation of latency in viral infected cells.

Considerable progress has been made in the area of the automation of cytology. Since most of the work has been done under a contract-supported program, it is reported in the contract section.

The Image Processing Unit (Dr. L. Lipkin, Head) conducts research in the reproducible characterization of cell morphology and kinetics, employing living as well as fixed and/or stained material as subjects for examination and measurement. Major attention during the year was devoted to the construction of software, optical and digital computer support for the real time role of the biologist in the characterization and measuring role. In view of the generality of many of the image analysis/processing algorithms, and the device independence of much of the hardware, a very large proportion of the work of the Unit bears directly on such diverse fields as electron microscopy, ultrasonics, nuclear medicine and radiology.

The Automatic Cell Analysis project involves the construction of a fully computer-controlled microscope, a special purpose processor and a controlling mini-computer along with necessary monitor and debugging software, Real Time Picture Processor (RTPP), and of an artificial intelligence-like system resident in the DCRT PDP-10, embodying a procedural description language and facilities for on-line modeling of cell descriptions and control facilities for RTPP. The system is intended to perform real time measurements and characterization of living cells as well as fixed and stained material. In fact, the ability to track a cell and its direct descendants through several successive cell cycles is a major reason for constructing a system of such complexity. During the year, construction of the buffer memory system was

completed. The subsystem is operational and is being debugged. It will be installed at the Image Processing Unit prior to the end of the fiscal year. Items 1, 3, 4, 6, 7 and 8 are already fully constructed and interfaced to the PDP8/e. Thus, with the completion of the buffer memory system, work on living cell systems may begin as planned for FY 1977.

Extensive time lapse cinematographic and culture studies of the macrophage line P388D were carried out during the year, since it is to be used in the first experiments with computer-controlled microscopic observations of living material. Much attention was devoted to cell cycle time and related studies.

CONTRACT RESEARCH PROGRAM

The Division of Cancer Biology and Diagnosis supports three separate program thrusts through contract mechanism. This includes the Immunology Program organized into Immunobiology, Immunodiagnosis and Immunotherapy; Breast Cancer Task Force with four separate areas of interest: Diagnosis, Epidemiology, Experimental Biology and Treatment; and Cancer Diagnosis Program, which consists of general Diagnosis research, Diagnostic Radiology and Cytology Automation. Cancer Diagnosis Program also includes two screening projects: Breast Cancer Detection Demonstration and Lung Cancer Screening.

The largest effort is in the area of Tumor Immunology, managed by the office of the Associate Director for Immunology. The program consists of three subunits: immunobiology, immunodiagnosis, and immunotherapy. Although classified this way, some of the projects naturally cross these delineation boundaries. Basically, the goals are (1) using immunologic techniques, to detect agents etiologic to cancer; (2) to prevent cancer spread by immunologic manipulation; (3) improve diagnosis of cancer by developing immunologic methodology; and (4) manipulate the immune system of patients with established cancer to achieve increased survival. Specifically, research in immunobiology concentrates on identification and characterization of surface membrane components, investigation of host reactivity and development of special cell lines. The immunodiagnosis effort leans toward the search for new tumor associated antigens in human cancer, evaluation of carcinoembryonic and other fetal antigens as diagnostic tools and evaluation of serologic assays, humoral and cellmediated immune response to tumor associated antigens. In the therapy area, the work is mostly preclinical, but human studies are being conducted with such agents as BCG and MER.

Breast Cancer Task Force has undergone major changes in the past year. New, non-NCI chairmen were appointed for all technical review committees, the membership of the Steering Committee was expanded to include representatives from all NCI divisions and the frequency of meetings was increased substantially. The BCTF continues to focus its sights on determining the etiology of the disease; identifying high risk populations; improving methods of diagnosis, particularly of small lesions; finding new treatment modalities; and preventing the occurrence and recurrence of the disease. To meet these goals the Task Force contract program considers the totality of breast cancer both in women and in the experimental animal, through studies related to seven subject areas to be enumerated below. Some contract projects contribute research in more than one category.

Seven basic categories can be identified in the Program of Breast Cancer Task Force:

I. Genetic, environmental, dietetic and other factors in breast carcinogenesis.

II. Pathological history of mammary gland and risk of neoplastic trans-

formation.

III. Isolation, cultivation, and characterization of normal and neoplastic mammary cells of epithelial origin.

IV. Intrinsic and extrinsic factors affecting the defenses of the host and growth potential of mammary carcinoma.

V. Metastases: Characterization, marking, measurement and control.

VI. Physical tools in breast cancer diagnosis.

VII. Dependency and responsiveness of mammary cells to hormones.

The Cancer Diagnosis Program covers five broad categories of research: general cancer diagnosis, diagnostic radiology, cytology automation, breast cancer diagnosis and immunodiagnosis. The last two areas are administratively in other organizational units and have already been reported.

One of the oldest projects is the screening of high risk individuals for lung cancer, in which three institutions cooperate in entering study cases. Additional cooperative studies are carried out in the areas of diagnosis for cancer of the pancreas and gastrointestinal cancer (methods of bowel preparation prior to barium enemas, CEA analysis, detection of human blood in stool specimens). In the realm of physical detection tools, NMR is being studied.

In diagnostic radiology, the improvement of x-ray imaging was of great concern. Five institutions are involved in evaluating the equipment for computer-assisted tomography of the brain and two projects are devoted to investigating the use of proton beams in tissue density measurements. Seven additional projects are concerned with various aspects of diagnosis by computerized transaxial tomography.

The Cytology Automation Program has concentrated on evaluation of sensing-screening and decision-diagnostic systems as well as on development of new techniques for specimen collection and preparation.

LABORATORY OF BIOCHEMISTRY

I. INTRODUCTION

II. BIOCHEMISTRY OF GENE EXPRESSION SECTION (Dr. E. B. Thompson, Head)

- A. Use of Hybrid Cells to Study the Mechanism of Response to Steroid Hormones.
- B. Purification of Hormone Receptors.
- C. Use of "Cybrids" to Study Induction by Steroid Hormones.
- D. Clinical Studies on Steroid Hormones and Cancer.
- E. Miscellaneous (Xeroderma Pigmentosum, NMR Studies, Sterol Receptors and Induction of C-Type Virus).

III. BIOSYNTHESIS SECTION (Dr. E. L. Kuff, Head)

- A. Collagen Synthesis.
- B. DNA Polymerases.
- C. Cellular Interactions in the Immune Response.
- D. Structures of Macromolecules.
- E. Intracisternal Type-A Particles.
- F. SV40 Carrying Host DNA.

IV. CELL PHYSIOLOGY AND ONCOGENESIS SECTION (Dr. K. Sanford, Head)

- A. Effects of Benzo(a)pyrene on Epithelial Cells in Tissue Culture.
- B. Correlation Between Cell Shape and Tumorigenicity.
- C. Methylation of Eukaryotic DNA.
- D. Effects of Light and Oxygen on Karyotypic Stability and Cell Growth in Tissue Culture.
- E. Correlation Between Surface Topography and Neoplastic Transformation in Tissue Culture.
- F. Diagnosis of Neoplastic Transformation in Tissue Culture.
- G. Studies on Serum Substitutes for Cells in Tissue Culture.
- H. Effects of BCG-Activated Macrophages on Neoplastic and Nonneoplastic Cells in Tissue Culture.
- I. Cellular Cytoskeleton of Neoplastic and Nonneoplastic Cell Lines.
- J. Growth in Soft Agar if Neoplastic and Nonneoplastic Cell Lines.

V. CELLULAR REGULATION SECTION (Dr. R. F. Goldberger, Head)

- A. Regulation of Expression of the Histidine Operon of Salmonella typhimurium.
- B. Effect of Estrogen on Gene Expression in the Avian Liver.

VI. NUCLEIC ACID ENZYMOLOGY SECTION (Dr. M. F. Singer, Head)

- A. Polynucleotide Phosphorylase.
- B. Cyclic AMP Phosphodiesterase.
- C. Structure of Host-Substituted SV40.

D. SV40 Capsid Protein.
E. Interaction of H1 Histone with Superhelical DNA.
F. Effect of SV40 Infection on Host Histone Synthesis.

VII. PROTEIN CHEMISTRY SECTION (Dr. E. Peterson, Head)

A. Chromosome Transfer.
B. Granulocyte Development.
C. Cellular Immune Reaction: Separation of B-Cells from T-Cells.
D. Membrane Proteins in Transformed Cells and in Hybrid Cells.
E. Displacement Chromatography of Proteins.

SUMMARY

Annual Report of the Laboratory of Biochemistry, National Cancer Institute
July 1, 1975 through June 30, 1976

I. INTRODUCTION

The Laboratory of Biochemistry (Dr. R.F. Goldberger, Chief) is divided into six Sections. The Biochemistry of Gene Expression Section studies control of gene expression in eukaryotic cells and its application to clinical medicine. The effects of steroid hormones on enzyme induction and cell function in tissue culture are investigated, along with steroid receptors in cells from patients with leukemia and breast cancer. The Biosynthesis Section is concerned with the relationship between cell function and organization at the molecular level. Biochemical, tissue culture, cell fractionation, and electron microscope techniques are employed. In the Cell Physiology and Oncogenesis Section the major objective is to elucidate the mechanisms of so-called "spontaneous", virus-induced, and chemical carcinogen-induced malignant transformation in cultured cells. Research toward this objective includes studies on cell growth and nutrition, cytology, ultrastructure, immunology and biochemistry. The Cellular Regulation Section studies regulation of gene expression in eukaryotes at the molecular level. The central problem under investigation is the mechanism by which estrogen induces the synthesis of egg yolk phosphoprotein in rooster liver, with special emphasis on the molecules involved in the interactions of hormone, hormone receptor, RNA polymerase and chromatin that result in the synthesis of transcripts of the phosphoprotein gene. The Nucleic Acid Enzymology Section is concerned with detection and study of enzymes involved in nucleic acid synthesis and degradation. Systems under investigation include DNA enzymes related to simian virus 40 replication, structure of host-substituted simian virus 40 DNA, interaction of histones with DNA, bacterial restriction endonuclease, and bacterial polynucleotide phosphorylase and cyclic AMP phosphodiesterase. The Protein Chemistry Section emphasizes the physical, chemical, and biochemical aspects of proteins and subcellular particulates involved in the synthetic, control, and differentiation systems of organisms. Problems currently under study include transfer of isolated chromosomes to mammalian cells, granulocyte development, fractionation of cells involved in cellular immune reactions, and techniques for fractionation of proteins.

II. BIOCHEMISTRY OF GENE EXPRESSION SECTION (Dr. E. B. Thompson, Head)

A. Use of Hybrid Cells to Study the Mechanism of Response to Steroid Hormones. A major part of the effort of the Biochemistry of Gene Expression Section has been devoted to the use of somatic cell hybrids to try to define the nature of cellular controls over steroid-evoked responses. From earlier studies it is known that glucocorticoid induction of tyrosine aminotransferase is blocked in hybrids between inducible and non-inducible cells. The gene responsible for this "repressive" effect has tentatively been assigned to the human X chromosome. Now under study are hybrids designed to answer the following questions: 1) Are the inhibitory effects of steroids on some cells also recessive in hybrids? 2) Are the inductive responses to steroids of different classes inhibited in hybrids? 3) Does each of the various specific inductions by a given hormone have its own corresponding repressor function? Preliminary results indicate that the answer to all of these questions in the systems studied is yes. If this is so, the current models for the mechanism of steroid action do not afford an explanation.

Future experiments with hybrid cells are designed to allow identification of the human chromosome carrying the gene for the glucocorticoid receptor and to confirm or deny the assignment of the repressor for tyrosine aminotransferase to the X chromosome. For this purpose a line of human leukemic blasts will be utilized. These cells are lysed by glucocorticoids at low concentrations. They will be employed to study, both at the cellular and biochemical level, the lympholytic action of these hormones. This is a difficult and complex problem, much investigated but never solved in rodent cells; and it has hardly been examined in human lymphoid cells.

B. Purification of Hormone Receptors. Studies are in progress on purification of the glucocorticoid receptors. The findings indicate that there are two forms of this activity by chromatography on DEAE-cellulose columns. This similarity with the early stages of purification of the progesterone receptor reported in the litterature has led to an examination of the two forms of the glucocorticoid receptor to determine whether their DNA/chromatin binding activities are similar to those reported for the receptor for progesterone.

C. Use of "Cybrids" to Study Induction by Steroid Hormones. In studies on the control over steroid-induced events, "cybrids" between enucleated L cells (which were selected for cytoplasm-borne chloramphenicol resistance) and whole T3C1-2 erythroleukemic cells showed a dramatic reduction in induction of hemoglobin by dimethylsulfoxide. The molecular mechanism of this finding is under study. As an off-shoot of these experiments, the observation was made that treating HTC or FU55 hepatoma cells with concanavalin A resulted in the very rapid, reversible inactivation of intracellular tyrosine aminotransferase. Studies of this unusual effect of a lectin are nearly complete.

D. Clinical Studies on Steroid Hormones and Cancer. Work on clinical material continues to go well. Biopsy specimens from over 400 breast-cancer patients have been examined for the presence of four classes of steroid receptors. The data are now being collated and compared with the clinical course and treatment of these patients. A prospective protocol for stratified, randomized trial of hormonal therapy, based on the results of receptor studies in breast cancer,

has been approved by the Treatment Committee at NIH. Cellular specimens from leukemic patients have, of course, been slower in coming, but data on the glucocorticoid responses of these cells is also being accumulated.

E. Miscellaneous (Xeroderma Pigmentosum, NMR Studies, Sterol Receptors and Induction of C-Type Virus). In their early stages are several small projects which will be continued during the coming year. These include mutagenesis of xeroderma pigmentosum cells by UV light, nuclear magnetic resonance studies of whole cells, identification of receptors for sterols, and the mechanism of the glucocorticoid augmentation of C-type virus induction by halogenated base analogues.

III. BIOSYNTHESIS SECTION (Dr. E. L. Kuff, Head)

A. Collagen Synthesis. Dr. B. Peterkofsky and her associates, continuing their study of collagen synthesis in normal and transformed cells, have compared the submicrosomal localization of prolyl and lysyl hydroxylases, enzymes that catalyze hydroxylation of the respective amino acids in nascent procollagen chains. Although 90-93% of the prolyl hydroxylase was found to be intracisternal, only half of the lysyl hydroxylase was, the rest being tightly bound to the microsomal membrane. The functional significance of this difference in distribution of two enzymes with similar functions is being investigated. Studies have also continued on identification of a reducing cofactor for prolyl hydroxylase in Kirsten virus (MSV)-transformed BALB/c 3T3 cells (Ki-3T3). These cells, in which proline hydroxylation is ascorbate-independent, contain reduced pteridine which can act as reductant for the enzyme in vitro. There is approximately 7-fold more pteridine in the transformed cells than in the ascorbate-dependent parent line, suggesting that a pteridine may be the active cofactor in Ki-3T3 cells. The relative rate of collagen synthesis in Ki-3T3 cells is about one-third that of the parent line and a different genetic type of collagen is synthesized. Thus, viral transformation in these cells has been accompanied by alteration in the genetic expression of collagen synthesis.

B. DNA Polymerases. Dr. Wilson and his colleagues have pursued their study of mouse myeloma DNA polymerizing enzymes, concentrating in the past year on the alpha class of DNA polymerase (previously DNA polymerase I). Three enzymatically active species of this enzyme were separated by DEAE-cellulose chromatography and individually purified. All three had similar sizes and catalytic properties. Two of the species have been analyzed by polyacrylamide gel electrophoresis. Both contained a major polypetide with a molecular weight of 120,000; however, other polypeptides in the two species differed in size. Multiple enzyme species of alpha class polymerase have also been observed in extracts of fetal bovine liver. To investigate the mechanism of action of the enzyme, the template-primer specificity of purified myeloma polymerase was studied using various combinations of DNA and RNA homopolymers. Marked variations in activity were found. By far the highest activity was observed with poly(dT) as template and oligo(rA) as primer; this combination was also two to four times more effective than maximally activated calf thymus DNA itself. No primer specificity was detected with calf thymus DNA; its replication was effectively primed by either DNA or RNA, and in the latter case all four ribonucleotides were present at the RNA-DNA joints. However when replication of single-stranded calf thymus DNA was studied after various template-primer complexes had been attached to its 3'-terminus, only the

poly(dT)·oligo(rA) hybrid initiated replication. Alpha polymerase reaction products are synthesized in the form of short chains, with an average length of 25 nucleotides; the enzyme appears to be self-limiting with regard to chain elongation.

C. Cellular Interactions in the Immune Response. Dr. Shelton, together with Dr. Orenstein, has continued a study aimed at detection and characterization of cell interactions in the immune response. Lymphocytes, macrophages, and mast cells of the mouse peritoneal cavity have been examined by light and scanning electron microscopy. Criteria for the "normal" appearance of the cell surfaces were established by comparison of cells fixed in situ with those fixed after being allowed to settle on artificial substrates. In the course of this work, some micro-anatomical features of the mouse mesenteries and omentum were elucidated. A study of metabolic factors that may regulate the surface structures of lymphocytes was initiated. When the cells were examined after treatment with a variety of inhibitors, exposure to iodoacetamide ($5x10^{-3}$M) and to low temperature (4^{o}) were found to induce characteristic changes in surface configurations.

D. Structures of Macromolecules. In a different direction, Dr. Shelton has also continued to study the structures of macromolecules using high-resolution transmission electron microscopy. Within the past year, she has collaborated with investigators from other laboratories and institutions to examine: a) the C5b-9 complement complex that binds to cell membranes and induces lysis; b) the C-reactive protein (CRP) which increases in serum during inflammation and binds specifically to human T-lymphocytes; and c) a major cell surface protein (CSP) from chick embryo fibroblast. The first two were shown to have complex subunit arrangements in circular (C5b-9) or radial (CRP) configuration; CSP appeared to be a filamentous structure.

E. Intracisternal Type-A Particles. During Dr. Kuff's absence for part of the year, Drs. Lueders and Segal continued studies on mouse tumor-associated intracisternal type-A particles. Dr. Lueders completed investigations which show that the particles are dynamic structures, undergoing a cycle of synthesis, assembly and decay in association with the internal cellular membranes. No evidence has been obtained for an extracellular phase. Dr. Segal has shown that the isolated particles contain polyadenylated RNA that directs polypeptide synthesis in a cell-free system. The informational content of this RNA is under investigation.

F. SV40 Carrying Host DNA. Since his return, Dr. Kuff has initiated a project dealing with transcription of covalently-linked cellular and SV40 viral DNA. The work is related to and extends work done with Dr. Ernest Winocour of the Weizmann Institute of Science during Dr. Kuff's working assignment there. The investigation is based on the fact that SV40 DNA becomes covalently integrated into host cell DNA during transformation (and probably during lytic infection as well) and considers the possibility that this association could have important effects in regulating expression of both host and viral genetic information. The problem is being approached by studying the in vivo transcription of defective substituted SV40 variants, in which parts of the closed circular viral DNA have been deleted and replaced with covalently linked host cell DNA.

IV. CELL PHYSIOLOGY AND ONCOGENESIS SECTION (Dr. K. Sanford, Head)

A. Effects of Benzo(a)pyrene on Epithelial Cells in Tissue Culture. Dr. Fox and Mr. Price, in collaboration with Dr. Selkirk (CB, NCI) demonstrated that primary cell cultures derived from human skin epithelium metabolize benzo(a)pyrene (BP) to three classes of compounds: phenols, quinones, and dihydrodiols. The relative proportion of these metabolites varied according to the skin donor but differed from the metabolite pattern in rat liver microsome preparations. The epidermal cells were killed by the treatment with BP. Cultures of fibroblasts from the same skin samples as the epithelial cells metabolized the hydrocarbon to a different extent and showed none of the cytotoxic effect observed with the epidermal cells. Cultured human prostate cells also metabolized BP efficiently in a manner similar to the epidermal cells. These findings that the differential capacity of epithelial cells to metabolize potential carcinogens, a capacity reduced in fibroblasts or other nonepithelial cells, were interpreted by Dr. Fox et al. to suggest that they may account for the increased susceptibility of epithelial tissue to neoplastic disease in humans. These results also emphasize individual differences in BP metabolite profile.

B. Correlation Between Cell Shape and Tumorigenicity. In collaborative studies with Drs. Dvorak (LPD, NIAID), and Dr. Caspersson (Karolinska Institute, Stockholm) Drs. Fox and Sanford have been quantifying cytometric parameters of potential importance for neoplastic transformation in mammalian cell populations in vitro. An initial study has demonstrated a close correlation between rounding of cells at metaphase and tumorigenic potential. This phenomenon may reflect a fundamental difference between nonneoplastic and neoplastic cells initially derived from the same cell in vitro. Mechanisms to account for this difference are under study.

C. Methylation of Eukaryotic DNA. Dr. Gantt and his colleagues have developed a procedure for purifying RNA methylases and a method, using pressure chromatography, to determine 5-methylcytosine in small amounts of DNA without the use of radioisotopes. The latter technique will provide information for understanding the biological function of eukaryotic DNA methylation. Selected methylases are being purified for antibody preparation for single cell fluorescent assays and quantitation in small numbers of cells. Work has continued in two areas: 1) examination of the distribution of 5-methylcytosine in chromatin fragments, to see if the distribution is non-random; and 2) efforts to establish whether mitochondrial DNA contains 5-methylcytosine.

D. Effects of Light and Oxygen on Karyotypic Stability and Cell Growth in Tissue Culture. Dr. Sanford and Mr. Price, in collaboration with Dr. Parshad (Harvard Medical School) have shown that both light and oxygen are factors that influence karyotypic stability of mouse cells in culture. Exposure of cells to room lights (cool white fluorescent) during routine handling increased the incidence of abnormal chromosomes that originate from chromatin breaks during interphase. A rapid assay for DNA strand breaks and linkage, in collaboration with Dr. Kohn (LMP, NCI) is currently in use to look for factors in the culture medium, particularly photodynamic reactions, that enhance or prevent damage to DNA and chromosomes. The possible relationship of the effects of light and oxygen on chromosome stability with "spontaneous" transformation is under investigation.

Dr. Taylor has been analyzing the relationship between measured pO_2 of the culture medium, inoculum size, and colony number or population size, with the ultimate objective of analyzing the mechanism of oxygen effect on cells in culture. Whereas growth of sparsely populated cultures is inhibited by oxygen at concentrations exceeding a pO_2 of 40 mm Hg, growth of mass cultures is inhibited little or not at all. These findings raise the question of whether oxygen regulates cell proliferation or causes the production of cytotoxic products, such as H_2O_2. Glucose utilization and lactate production were found to vary inversely with the inoculum size and oxygen concentration.

E. Correlation Between Surface Topography and Neoplastic Transformation of Cells in Tissue Culture. Studies on the surface topography, with Dr. Wetzel (DB, NCI), and cell coat of paired neoplastic and nonneoplastic cell lines in culture were completed and showed that cells of neoplastic lines were characteristically less spread and displayed greater variability in surface features but that the incidence of microvilli, ruffles and blebs did not prove reliable criteria of the neoplastic state.

F. Diagnosis of Neoplastic Transformation in Tissue Culture. Mrs. Handleman and Dr. Sanford identified cytologic characteristics of rat liver parenchymal cells that permit accurate diagnosis of neoplastic transformation in vitro. In collaboration with Dr. Lipkin (DCBD, NCI) a computerized image analysis procedure has been used to quantify the orientations of cells migrating from a concentrated cell suspension, a parameter of colony morphology particularly useful in determining neoplastic state of fibroblasts in culture.

G. Studies on Serum Substitutes for Cells in Tissue Culture. In further efforts to analyze the peptide-like growth factor in the autoclavable, low molecular weight fraction of commercial peptone (PD), Dr. Taylor found that Chelex-100 (Na^+) chromatography resulted in a 1.3- to 1.4-fold purification, as measured by cell yields in chemically defined medium with treated and untreated PD. The tripeptide glycylhistidyllysine was tested but failed to substitute for PD.

H. Effects of BCG-Activated Macrophages on Neoplastic and Nonneoplastic Cells in Tissue Culture. Dr. Tucker has shown that the susceptibility of long-term cell lines to killing by BCG-activated macrophages is directly related to the tumorigenic potential of the cells (as measured by tumor latent periods in irradiated syngeneic hosts). The differential susceptibility was observed in paired nonneoplastic and neoplastic cell lines of diverse species. Some pairs did not differ in surface morphology, growth rates or saturation densities in vitro, properties often considered "markers" of neoplastic state. Two human choriocarcinoma cell lines were the first neoplastic lines found to be resistant to killing by BCG-activated macrophages. This resistance appeared to be associated with inability of the activated macrophage to come into intimate contact with the surface of the neoplastic cells. The increased translational motion of BCG-activated macrophages in attacking neoplastic cells was neither necessary nor sufficient for killing. This conclusion was based on the findings that cortisone inhibited killing without altering the motion, cytochalasin B inhibited killing with a decrease in motion, and concanavalin A stimulated killing in the absence of increased motion.

I. Cellular Cytoskeleton of Neoplastic and Nonneoplastic Cell Lines. A comparison of seven different pairs of nonneoplastic and neoplastic cell lines with respect to cytoskeleton was carried out on fixed cells with fluorescent antibodies to actin and tubulin and on living cells by polarization microscopy. Cells of nonneoplastic lines showed extensive cytoplasmic microtubules and thick actin cables, whereas those of the neoplastic lines showed fewer actin cables and microtubules were visible only in well-spread cells. The visualization of actin cables and microtubules was directly related to the degree of cell spreading, whether cells were neoplastic or not.

J. Growth in Soft Agar if Neoplastic and Nonneoplastic Cell Lines. Dr. Tucker and Dr. Sanford found with paired cell lines that nonneoplastic cells did not produce colonies in semi-solid agarose, while neoplastic cells produced colonies the number and size of which were not related to tumorigenicity as measured by latent periods for tumor development *in vivo*. This is one of the few studies on spontaneous transformants, and the results showed plating efficiencies in semisolid medium that were conspicuously lower than those reported for virally transformed cells.

V. CELLULAR REGULATION SECTION (Dr. R.F. Goldberger, Head)

A. Regulation of Expression of the Histidine Operon of Salmonella typhimurium. This project was not under investigation this year, but is briefly discussed here because a small amount of time was spent writing up the results of the previous year's studies.

In one study, mutants were isolated in which a single mutation in the first structural gene of the histidine operon was shown to produce two different effects: 1) inability of the enzyme specified by the first structural gene to be inhibited by the amino acid, histidine; and 2) a *trans* recessive regulatory defect resulting in constitutive expression of the histidine operon. The conclusion from these results was that the enzyme specified by the first structural gene of the histidine operon plays a dual role in histidine biosynthesis, acting both as an enzyme that catalyzes one of the steps in the histidine pathway and as a regulatory protein that influences expression of the operon as a whole.

In another study, it was demonstrated that the enzyme specified by the first structural gene of the histidine operon interacts with the DNA of the histidine operon. Interaction of pure enzyme with the DNA of a defective $\phi 80$ transducing bacteriophage carrying the histidine operon in its genome was studied under a variety of conditions. In addition, DNA of several different phages, each carrying a slightly different histidine operon, allowed more detailed analysis of the site within the histidine operon at which the enzyme binds. The findings showed that the enzyme binds with a high degree of specificity to a site within or very near the regulatory region of the histidine operon. These results are consistent with the growing weight of evidence that indicates that the enzyme specified by the first structural gene of the histidine operon plays a role in regulating expression of the operon.

B. Effect of Estrogen on Gene Expression in the Avian Liver. Several projects on this topic involve an investigation of the mechanism by which estrogen induces the synthesis of vitellogenin, the precursor of the egg yolk

phosphoproteins, in rooster liver. Vitellogenin is normally synthesized in the liver of the laying hen and is transported through the plasma to the ovary, where it is deposited in the developing oocyte. Immature chickens and roosters do not normally synthesize vitellogenin. Following an injection of estrogen, however, vitellogenin synthesis is induced in these animals. Using a rabbit reticulocyte cell-free system to monitor the mRNA that specifies vitellogenin, this mRNA has been partially purified and has been shown to have a molecular weight of approximately 2×10^6 daltons. Vitellogenin mRNA was found to be absent from normal rooster liver, whereas it was found to be synthesized and to accumulate in the livers of roosters that had been stimulated with estrogen. Thus, induction of vitellogenin synthesis in the rooster is regulated, at least in part, at the transcription level, by a positive effect of estrogen on expression of the vitellogenin gene.

A particularly interesting observation that was made recently is that after the estrogen response had apparently died away and the rooster was no longer synthesizing vitellogenin (post-estrogen-serum-negative state), vitellogenin mRNA was still present in the liver. This mRNA, which is of the same size as that found in the estrogen-stimulated, vitellogenin-synthesizing rooster, was found to be present at a concentration approximately 30% that found under conditions of maximal stimulation. A difference between the vitellogenin mRNA found in the liver of the vitellogenin-synthesizing rooster and that found in the liver of the post-estrogen-serum-negative rooster is that in the former case it is associated with large polysomes whereas in the latter case it is not associated with polysomes at all. It appears likely that the vitellogenin mRNA of the post-estrogen-serum-negative rooster is present as a mRNP. The working hypothesis is that vitellogenin mRNA in this mRNP is recruited by the translation system into polysomes after administration of a second dose of estrogen, accounting, at least in part, for the increased rapidity, magnitude and duration of the secondary estrogen response. If this is correct, then vitellogenin synthesis is regulated not only at the level of transcription, but also, under certain conditions, at the translation level.

VI. NUCLEIC ACID ENZYMOLOGY SECTION (Dr. M. F. Singer, Head)

A. Polynucleotide Phosphorylase. After literally years of confusion and frustration among nucleic acid enzymologists, Dr. Klee and Dr. Barbahenn are beginning to make sense out of the multitude of protein forms assumed by highly purified polynucleotide phosphorylase. It now appears that the presence or absence of heretofor unsuspected ligands is of primary importance in this phenomenon. This progress in structural chemistry will have bearing on interpretation of data concerning the mechanism of the initiation of polymerization. But most interesting for the latter problem has been the discovery by Dr. Craine that a low level of activity of the adenylate kinase type is associated with polynucleotide phosphorylase.

B. Cyclic AMP Phosphodiesterase. The high yields of homogeneous activator protein for cAMP phosphodiesterase that are obtained by Dr. Klee's new procedures have permitted development, in turn, of an excellent new purification of the diesterase itself. The availability of the two highly purified proteins has permitted rigorous examination of the regulation of activation of the diesterase, especially as regards the role of calcium ions and cAMP.

C. Structure of Host-Substituted SV40. By utilizing the very newest, and in many instances unpublished, techniques for the analysis of DNA fragments, Dr. Rao was able to complete the analysis of the host-substituted SV40 genome that has been of interest in the Nucleic Acid Enzymology Section. The molecule contains a four-fold repeat of a combined SV40-host fragment. The portion of the host-derived sequence that originates in host repetitive DNA has been localized, by *in situ* hybridization, on many monkey chromosomes, in both centromeric and noncentromeric positions, by Dr. Segal (Biosynthesis Section) and Ms. Johnson (Biochemistry of Gene Expression Section). These studies constitute the first direct demonstration of the existence of reiterated sequences in non-centromeric positions. Such a distribution has been proposed, on the basis of hybridization kinetics, by other workers. Work on the nucleotide sequence of the same fragment of host-reiterated DNA (140 base pairs long), isolated from defective SV40, has reached the stage at which our collaborator, Dr. Martin Rosenberg (Laboratory of Molecular Biology), and Dr. Segal are able to write a tentative sequence for 123 of the 140 base pairs. This preliminary sequence is very different from the simple repetitive sequences typical of eukaryote satellite DNA.

D. SV40 Capsid Protein. Several seemingly unrelated observations made by different people in the lab have coalesced into the hypothesis that two common wild type SV40 strains differ in their capsid proteins. Dr. Kay is continuing with this work.

E. Interaction of H1 Histone with Superhelical DNA. Dr. BinaStein has elegantly confirmed Dr. Vogel's earlier conclusion that the preferential binding of H1 histone to superhelical DNA is not driven by an unwinding of the DNA duplex. Thus, the hypothesis that H1 histone actually interacts with a twisted duplex, or a site where two duplex chains are very close together, has been strengthened. This work also led to the observation that H1 histone inhibits the action of "unwinding enzyme", most likely by protecting the DNA. Dr. BinaStein has also demonstrated that H5 histone, the special lysine-rich histone of avian erythrocytes, has the same specificity for superhelices as does H1 histone. Dr. D. Singer has shown that the central, hydrophobic and globular region of H1 histone is important for the recognition of superhelices while the lysine-rich, carboxy-terminal portion of the molecule is important for binding to DNA *per se*. Phosphorylation of H1 histone at residue 37 or residue 105, or a combination of 4 other residues in the carboxy- and amino-terminal portions of H1 histone, has little effect on recognition of superhelices at moderate salt concentration (0.1 M). However, at low ionic strength, interesting differences in specificity can be seen. These experiments are being carried out in collaboration with Prof. T. Langan, University of Colorado, who prepared the phosphorylated H1 histone.

F. Effect of SV40 Infection on Host Histone Synthesis. Dr. Kay, has been studying the synthesis of histones upon SV40 infection. Base line data for a greatly stimulated rate of synthesis of all histones, related to viral DNA synthesis, have been obtained. Dr. Kay is now concentrating on aspects of the synthesis of H1 histone in particular.

VII. PROTEIN CHEMISTRY SECTION (Dr. E. Peterson, Head)

A. Chromosome Transfer. Dr. McBride and his colleagues have demonstrated the serial transfer of a human gene for hypoxanthine phosphoribosyltransferase to mouse cells and then to Chinese hamster cells via two chromosome isolations

and uptake of chromosomes by mutant recipient cells. Only Chinese hamster chromosomes were found on karyotypic analyses of the final transformed hamster cells. These results indicate that genes transferred by chromosome uptake are integrated into the chromosomal DNA of the recipient cell.

The thymidine kinase (TK) in 12 independent transfer clones isolated after incubation of TK-deficient mouse cells with chromosomes from wild-type Chinese hamster cells has been identified as the donor species. Two of these clones also express the donor species of galactokinase. Back-selection of both colonies in BUdR for loss of TK resulted in the coordinate loss of the hamster (but not of the mouse) species of galactokinase, demonstrating that cotransfer of these two genes did occur.

Hybridization experiments employing a labeled DNA probe containing repetitive sequences found in the transformant but not in the parental mouse line indicate that a small fraction of the probe repetitive DNA is homologous with hamster DNA but not with mouse DNA. Preliminary estimates indicate that the transgenome represents no more than about 1/20 of a chromosome (about 4×10^6 base pairs).

B. Granulocyte Development. Dr. Evans and his co-workers are exploring the possibility of using the response of the bone marrow of the guinea pig to inflammatory stimuli as a model for studies of the regulation of granulocyte production. In preparation for this, the kinetics of cell changes in the marrow after intraperitoneal injection of sterile glycogen have been studied. Also, improved procedures for the extraction and resolution of several glycoproteins specifically associated with the cytoplasmic granules have been developed in anticipation of the use of these proteins as markers in following the differentiation of granulocyte precursors. Preliminary experiments have shown that these cells incorporate ^{14}C-leucine into all the major granule proteins.

Immature granulocytes were found to contain 3 to 4 times as much spermidine as mature cell preparations, and the incorporation of ^{14}C-ornithine into putrescine and spermidine in vitro was 5 to 6 times greater in immature cells than in mature cells. However, neither of the two cell preparations was able to convert ^{14}C-arginine to polyamines.

C. Cellular Immune Reactions: Separation of B-Cells from T-Cells. Dr. Mage, pursuing his interest in cells involved in cellular immune reactions, has developed a preparative method for separating spleen cell suspensions into cells with surface immunoglobulin (B-cells) and those without surface immunoglobulin (mostly T-cells). This is accomplished by incubating the suspension in petri plates coated with specifically purified anti-immunoglobulin. The separated B-cells and T-cells were both able to bind cells bearing graft-versus-host activity when tested as target cells.

D. Membrane Proteins in Transformed Cells and in Hybrid Cells. Dr. Marciani continued his investigation of changes in the surfaces of chick embryo cells transformed by a temperature-sensitive mutant of Rous sarcoma virus. He found that these cells develop an ability to adsorb red cells at 41°, the nonpermissive temperature for transformation, but lose this ability at 37°, the permissive temperature. The changes were reversible and did not

depend on RNA synthesis or protein synthesis. Treatment of the cells with phospholipase c or incorporation of 2-deoxy-D-glucose into the cells resulted in inhibition of the hemadsorption, suggesting that this activity may be due to properties of the lipid fraction of the membrane.

Dr. Marciani also examined the membrane polypeptides of 3 clones of somatic cell hybrids (isolated by Dr. E.B. Thompson) and the parent cells: mouse L cells and rat hepatoma cells. The hybrids were shown to have similar distributions of membrane polypeptides, but all had higher proportions of polypeptides of M.W. 56,000 and 45,000 than did the parent cells. The major glycoprotein in the hybrids resembled that of the parent rat cell and not that of the mouse. A large external protein (M.W. 240,000), identified by lactoperoxidase-catalyzed iodination, was missing in the parent cells but was present at high levels in all the hybrid clones. There was no correlation between these differences in membrane proteins and growth control in vitro or tumorigenicity. A similar lack of correlation was seen in studies of the agglutination of these cells by concanavalin A.

E. Displacement Chromatography of Proteins. Dr. Peterson has continued the development of procedures for the fractionation of proteins by displacement chromatography. By preparing the CM-dextran (displacer) affinity continuum in "sections", each of which represents a narrow range of affinity, to be pumped successively into the protein-separating column, a substantial portion of the function of the column is achieved and preserved in the preparation of the displacers. This diminishes the load on the column and also has the effect of significantly diminishing the concentration of displaced counter ions. A novel and very simple device has been constructed to permit preselected volumes of multiple "sections" to be pumped sequentially into the column automatically.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5210-08 LB

PERIOD COVERED
July 1, 1975 to July 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular controls over growth and inducible processes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. B. Thompson	Head, Section on Biochemistry of Gene Expression	LB NCI
OTHER:	T. V. Gopalakrishnan	Visiting Fellow	LB NCI
	Y. Sakaue	Visiting Fellow	LB NCI
	Y. Zubairi	Intergovernmental Transferee	LB NCI
	B. Paterson	Staff Fellow	LB NCI

COOPERATING UNITS (if any) D. Marciani & E. L. Kuff, LB, NCI; M. L. Lippman & D. Tormey, COP, NCI; A. Tashjian & P. Dannies, Harvard Medical School; A. Kandutsch & H. Chen, The Jackson Laboratory; L. Loriaux, RR, CH; L. Lyons, A. Andrews, J. Robbins, D, NCI; P. Schein, Georgetown U., R. Ader & J. Cohen, LCP, NIAMD.

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Biochemistry of Gene Expression Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	8	4

SUMMARY OF WORK (200 words or less - underline keywords)

The nature of cellular controls over responses to steroid hormones has been studied through somatic cell hybrids. Processes studied in these hybrids include induction of tyrosine aminotransferase, prolactin, growth hormone and glutamine synthetase and inhibition of nucleic acid uptake, cell growth and cloning efficiency. Steroid-resistant cell variants have been developed. Characterization and purification of glucocorticoid receptors are underway. Receptors for glucocorticoids, androgens, estrogens and progestins, have been studied for their correlation with treatment and disease in human leukemias and breast cancer. Growth control in cell hybrids has been studied and hybrid membrane proteins analyzed.

Project Description:

Objectives: Our broad objective remains the same, namely to understand the ways in which eukaryotes regulate gene expression. Specific systems have been investigated in pursuit of that goal. All these systems have derived from our deep interest in the mechanism of action of steroid hormones. In the course of our studies on this topic, we have made from time to time, chance observations which seemed of enough interest and relevance to the main question that they have been pursued. Although they have not always pertained directly to the question of steroid action, they have always had to do with current issues in control of eukaryotic cells. In addition, we have applied our knowledge of mechanism of action of steroid hormones to several clinical situations. Opportunities to make use of information from basic research in direct human care situations do not occur on a regular basis. We have been delighted, therefore, to be able to make a contribution from basic science in the Cancer Institute, to clinical problems. Our specific lines of research, their derivation, and their objectives follow.

The first objective has been to define, at the cellular level, the nature of cell controls over differentiated steroid responses. Somatic cell hybrid studies have shown that the induction of tyrosine aminotransferase by glucocorticoids is eliminated in crosses between TAT inducible and TAT non-inducible cells. We have three specific short-term objectives: one, to see whether the inhibitory effects of glucocorticoids on certain cells are controlled by positively or negatively acting cell processes; two, to see whether various specific steroid-induced products are controlled independently; and three, to see whether there is dominant negative control in differentiated cells over products induced by all classes of steroids. In every case we desire to know whether a correlation can be made between the presence of certain chromosomes and the control function studied. Other specific goals, closely related to the first objective are also being pursued.

Thus, our second objective has been to develop variants with respect to steroid responsiveness in a number of cell lines and to define their properties at the physiological and biochemical level.

The third objective has been to seek evidence for the presence of cytoplasmic factors exerting control over gene expression.

Because of certain similarities in the cellular actions of steroids and those of sterols, we have developed our fourth objective, to see whether receptor molecules exist for the oxygenated sterols, analagous to those for steroids. The most popular model for steroid action holds that these hormones act by binding to cytoplasmic receptors forming a complex which moves to the nucleus, there stimulating the accumulation of specific RNA products. The induction of C-type viruses by 5-iodo-2

deoxyuridine can be stimulated by glucocorticoids. Certain experiments in the literature and of our own suggest that this steroid effect may may not be produced by the above model. Our fifth objective, therefore, is to see whether this is so.

Sixth, we are trying to achieve partial purification and characterization of glucocorticoid receptor. The fully purified progesterone receptor has two subunits with different properties. We wish to see whether the same holds true for the glucocorticoid receptor. In the long run, we hope to see whether glucocorticoid receptors differ in different tissues.

Seventh, are our clinically related objectives. 1) We want to know the role of receptors for various steroids in human breast cancer and whether they offer predictive information useful in selecting therapy. 2) Similarly we want to know the predictive value of glucocorticoid receptors in human leukemias and certain related diseases. 3) We want to know whether nuclear magnetic resonance can be of use distinguishing the hormone responsiveness of normal and malignant cells. 4) We wish to know whether the estrogenizing side-effects of the diuretic steroid spironolactone and of the cardiac glycosides can be explained on the basis of their affinity for androgen receptors.

Eighth is an objective stemming from our interest in cell control of growth and mutagenesis. (See objective 2 and completed projects, below.) Using xeroderma pigmentosa cells, we are conducting mutagenesis and transformation experiments. The objective is to see whether UV light can increase mutagenesis and cause malignant transformation in XP cells in vitro.

Methods employed: These have largely been described in prior reports. Briefly, those for objectives 1-3 are somatic cell hybridization, chromosome analysis, standard steroid receptor assays, assays for specific steroid-induced products or responses such as tyrosine aminotransferase, prolactin release, growth hormone release, DNA synthesis, cloning efficiency, and so forth, mutagenesis and cloning of cells, and development of new cell lines with "selective markers," and hybridization of enucleated cells with whole cells.

For the fourth objective, sucrose gradient analysis of radiolabelled sterols bound to cell proteins is being employed.

For objective 5, besides various standard assays for steroid receptors, we are using enzymic assay of RNA-directed DNA polymerase and plan to use radioimmuno assays for viral proteins.

Objective 6 requires the use of DEAE chromatography, DNA-cellulose chromatography, hydrophobic chromatography, chromatin and DNA binding assays, and hydroxyapatite chromatography.

Objective 7 employs the use of a number of steroid receptor assays and of measurement of relaxation times in NMR studies.

Objective 8 employs the treatment of several specific XP cell lines with UV light and analysis of frequency of formation of resistance to ouabain or thioguanine. Later we will be looking for oncogenic transformation in vitro as well, on the basis of altered cell growth and morphology.

Major Findings:

1) With respect to the 1st objective, several results are emerging but not yet complete. Hybrids between rat HTC and mouse L cells, when first formed, are resistant to the inhibition of thymidine uptake normally seen in L cells after steroids. Clones of hybrids with reduced chromosome numbers display the usual L cell type of inhibition. Recently we have observed a a shift from no inhibition to inhibition in a clone maintained in continuous culture. We are now examining the chromosomes from this clone to see if the HTC chromosomes are reduced. We tentatively conclude that this inhibitory response to glucocorticoids is recessive in hybrids, just as are inductive responses. (Lyons)

A second set of hybrids, between L cells and GH3 cells, is proving interesting. GH3 cells make prolactin and growth hormone. Prolactin is induced by estrodiol and growth hormone by glucocorticoids. In addition prolactin is induced by TRH, a tripeptide active at the cell surface. L cells show none of these responses, and all responses are lost in fresh hybrids. One hybrid clone has shown return of prolactin induction by estradiol and its chromosomes are being analyzed. (Dannies, Tashjian)

These "in-progress" results we interpret as suggesting that in hybrid cells, dominant factors prevent expression of each steroid-specific response independently. The implication is that in differentiated cells, failure to show specialized responses to specific steroids is not due to the absence of positive factors, as has been suggested by others, but instead is due to specific, dominant repressors.

2) Our major effort in objective two has been to start the characterization of several lines of human lymphoblastic leukemic cells. These cells have been characterized by others as having thymus-derived cell properties. We have found several of them to be sensitive to killing by glucocorticoids. We propose to utilize them, therefore, as the first continuous human cell line killed by steroids in studying the mechanism of that effect. Already several sensitive

and resistant subclones have been isolated and are being analyzed. Flow microfluorimetry has shown that sensitive cells are stopped in the G1 phase of the cell cycle. Basic steroid receptor and karyological data are being obtained. These cells offer several advantages over the rodent lymphoid lines or freshly dispersed thymus cells currently in use for studying the lympholytic effects of corticosteroids. (Norman)

3) In pursuing our third objective, the question of possible cytoplasmic controls over nuclear function, technical problems have prevented our carrying out intended experiments on steroid induction processes. Therefore, we have turned to another widely studied induction system, Friend virus carrying, mouse erythroleukemia cells. First, as mentioned in last year's report, chloramphenicol resistant L cells were developed. These cells were enucleated and fused with the erythroleukemic cells. By growth in the presence of chloramphenicol, "cybrids" were selected. After 2 or more months' growth, these cybrid cultures were tested for hemoglobin induction by DMSO. Whereas about 50% of the parent erythroleukemic cells could be induced to form hemoglobin by DMSO, only a few % of the hybrids did so. This experiment has been repeated on 4 occasions, with the same result, suggesting that a cytoplasmic factor is responsible for the reduced hemoglobin production. (Gopalakrishnan)

An interesting response to concanavalin A, noted during our enucleation studies, has been investigated. We observed that hepatoma cells treated with Con A showed inactivation of their tyrosine aminotransferase within 5 minutes, with equally rapid reactivation when the known competitor for binding of the lectin, α methylmannoside, was added. Both inactivation and reactivation were achieved in the absence of protein or RNA synthesis. No inactivation was seen when the lectin was added to broken cells. When extracts of lectin-treated cells were mixed with extracts from control cells, no inactivation of control-cell enzyme was seen. Thus, the lectin seems to act in intact cells to reversibly and rapidly inactivate this soluble-phase enzyme. (Gopalakrishnan)

(4) Regarding objective 4, α25-OH cholesterol binding peak has been identified in L cell cytoplasm, and its function is being investigated. (Chen, Kandutsch)

(5) The examination of the mechanism of C-type virus enhancement is still in its preliminary stage. (Zubairi, Kuff)

(6) Purification of glucocorticoid receptors has lagged behind that of other steroid receptors because of their greater instability. We have entered this field only in the last year. However, we have begun to have some success. Most interesting has been the observation that there seem to be two forms of glucocorticoid receptor in liver, L cells and HTC cells distinguishable on DEAE cellulose chromatography. We are trying to see whether these have the same properties described for the progesterone receptor.

Proposed course of research

We proposed to continue our pursuit of the long-range objective, understanding gene expression, particularly in relation to steroid hormones. This means that certain of the projects listed above we plan to develop, while others we expect to complete and then leave.

We expect to continue to use somatic cell hybrids as well as other techniques of cell biology to study control of cell responses to hormones. We plan to complete our examination of the GH_3 x L cell and HTC x L cell hybrids during the next year. By developing new hybrids using the human leukemic cells, we aim to map the chromosome containing the gene for the glucocorticoid receptor. With these hybrids we also hope to confirm or deny the tentative location of the repressor for tyrosine aminotransferase induction as being on the human X-chromosome. We expect to overcome the difficulties mentioned already and complete a study of the cytoplasmic effects on the steroidal inductive and inhibitory processes.

The human leukemic cell line is to be developed for studies in other ways by rescuing and characterizing resistant clones and by examining the biochemistry of the glucocorticoid-induced lethal effect. Rodent cells have been studied extensively concerning this effect, but little has been done with human cells.

We hope to renew our use of cell-free systems in the study of steroid action, and are presently considering several biological systems in which these methods may be best utilized.

Our attempts to characterize and purify the glucocorticoid receptor will be continued.

We intend to carry out a limited study of C-type virus induction enhancement by glucocorticoids to see whether and how steroid receptors may be involved.

We will plan to continue the examination of receptors and steroid responsiveness in clinical tumor material. Most of the assays for these studies are done outside our lab, and therefore do not occupy a great deal of our experimental effort. We do contribute some assays, however, and are involved in collating the data. Most important, we regularly collaborate in planning and evaluating the research. We want to continue our participation in this work because we think it important. In addition to the large retrospective study ongoing, our proposal for a prospective study, evaluating the usefulness of steroid receptor data in breast cancer has been approved by the Treatment Committee and will soon be begun.

Projects to be completed and not pursued further include several self-limiting collaborations, including the work on oxygenated sterols, nuclear magnetic resonance, xeroderma pigmentosum cells, and spironolactone.

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6) Thompson, E. Brad, Perlin, E. and Tormey, D.: Steroid binding proteins in human male breast carcinoma tissue. Amer. J. Clin. Path. in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 366-06 LB

PERIOD COVERED: July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biosynthesis and assembly of intercellular structural components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Kuff	Head, Biosynthesis Section	LB NCI
	K. K. Lueders	Chemist	LB NCI
OTHER:	S. Segal	Visiting Fellow	LB NCI
	D. Marciani	Visiting Associate	LB NCI
	C. Prives	Investigator	Not NIH

COOPERATING UNITS (if any)

Virology Section
Weizmann Institute of Science, Israel

LAB/BRANCH: Laboratory of Biochemistry

SECTION: Biosynthesis Section

INSTITUTE AND LOCATION: DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying murine intracisternal Type-A particles to determine their biological significance in normal development and neoplastic transformation. We have shown that the particles are dynamic structures, undergoing a cycle of synthesis, assembly and decay in association with the internal cellular membranes. The isolated particles contain polyadenylated RNA which directs polypeptide synthesis a cell-free system. The informational content of this RNA is under investigation.

PHS-6040
(12-75)

Project Description:

Objectives: To study the synthesis and transport of individual macromolecules and their assembly into complex intracellular components; to study the process of neoplastic transformation in terms of these coordinated functions.

Methods Employed: Cell fractionation by differential and density gradient centrifugation, isolated and gradient fractionation of nucleic acids and nucleoproteins, electron microscopy of isolated nucleoproteins, in vivo and in vitro incorporation of isotopically-labelled precursors into protein and nucleic acid, chromatographic, immunochemical and electrophoretic characterization of specific proteins, tissue culture of normal and transformed cells, nucleic acid hybridization, immumnofluorescent staining.

Results: Studies during the past year have been concerned primarily with mouse intracisternal type A-particles.

1. Dr. Lueders has completed studies showing that the major A-particle structural protein is synthesized on membrane-bound polyribosomes in cultured neuroblastoma cells. We had previously shown that A-particles are not secreted from these (or other) cells and that their structural protein turns over intracellularly at a rate somewhat greater than the average of the other cell proteins. A-particles appear to be dynamic structures which undergo a cycle of synthesis and decay in association with the internal cell membranes.

2. Dr. Segal has shown that isolated A-particles contain polyadenylated RNA which directs synthesis of discrete polypeptides in a cell free system (the latter in collaboration with Dr. Carol Prives). It has not yet been possible to show that these protein products are related to the structural protein of the particles themselves but work in this direction is in progress.

3. Using the techniques of molecular hybridization, Dr. Lueders is currently examining the specificity of mRNA sequences associated with A-particles isolated from different sources.

Proposed course of Research: We will attempt to determine whether intracisternal A-particles contain a distinctive collection of genetic information, using both the molecular hybridization and cell-free translation systems. Future experiments will be determined by the outcome of this effort.

Publications:

Lueders, K.K., and Kuff, E.L.: Synthesis and turnover of intracisternal A-particle structural protein in cultured neuroblastoma cells. J. Biol. Chem. 250: 5192-5199, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 379-15 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Ultrastructural characterization of macromolecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Emma Shelton	Research Biologist	LB NCI
OTHER:	Jan M. Orenstein	Senior Surgeon	LB NCI
	I. Pasten	Chief, Lab. of Molec. Biol.	LMB NCI
	K. Yamada	Research Associate	LMB NCI
	H. J. Muller-Eberhard		Not NIH
	W. P. Kolb		Not NIH
	H. Gewurz		Not NIH
	R. A. Lazzarini	Research Chemist	LMB NINDS

COOPERATING UNITS (if any)

Scripps Clinic and Research Foundation, La Jolla, Ca.
Rush Medical College, Chicago, Ill.

LAB/BRANCH Laboratory of Biochemistry

SECTION Biosynthesis Section

INSTITUTE AND LOCATION DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

SUMMARY OF WORK (200 words or less - underline keywords)

To relate the fine structure of macromolecules to their biochemical and physical properties, and to their function in biological systems.

PHS-6040
(12-75)

Project Description:

Objectives: To relate the fine structure of macromolecules to their biochemical and physical properties, and to their function in biological systems.

Methods and Major Findings:

1. Ultrastructure of complement components, C5-9; the "membrane attack complex".

Kolb and Muller-Eberhard have shown that upon activation of complement component, C5, a stable C5b-9 complex is formed. This complex has a transient ability to bind to cell membranes and cause lysis. The complex, without cytolytic activity remains in free solution. We are examining both the complex itself and its individual components, e.g., C6, C7, C8 and C5b-6, from human and rabbit serum. The complex appears to be composed of circular grouping of components (floret) attached to a "tail". The length of the complex is 32 nM; the width of the tail is 8 nM and the width of the "floret" is 13 nM. It is of interest that the width of the "floret" is compatible with the width of the circular lesion observed after complement lysis of erythrocyte membranes.

2. Ultrastructure of C-reactive protein (CRP).

C-reactive protein (first described in 1930 and later crystallized by McCarty in 1947) is a component of normal serum that increases dramatically in concentration during inflammation. It mediates many of the in vitro reactions that are also characteristic of immunoglobulins, but it clearly differs from immunoglobuoins in antigenicity, tertiary structure, and molecular heterogeneity. Gewurz has shown that human CRP binds specifically to human T-lymphocytes. We have examined the ultrastructure of CRP and find it to be composed of 6 radially-arranged subunits whose diameter is 10.8 nM. These may be stacked in a doublet, providing the complete molecule with a total of 12 subunits.

3. Ultrastructure of CSP (Cell Surface Protein).

Yamada has isolated from chick embryo fibroblasts a cell surface protein that constitutes up to 3% of the total cell protein. The CSP appears after negative staining to be a filamentous protein of undetermined length and width.

4. Ultrastructural studies of rhabdo- and paramyxo- viruses.

A continuing effort is being made to decipher the arrangement of the nucleocapsid and proteins within the whole vesicular stomatitis virus. In addition, factors that affect the packing of the nucleocapsid of rhabdo- and paramyxo-viruses are being examined. Dialysis of VSV capsids against

1 M Ca++ causes the nucleoprotein to form a tightly packed coil (termed "herring-bone") similar to that seen in native measles virus, a rhabdovirus.

Significance to Cancer Research: A knowledge of the structure of macromolecules obtained by observations with the electron microscope provides confirmation of physico-chemical data and, in many cases, an unequivocal basis for knowledge of their function.

Proposed Course of Research: To elucidate further the structure of macromolecules and correlate their structure with their function.

Publications:

Schubert, K.R., Switzer, R.L. and Shelton, E.: Studies of the quarternary structure and the chemical properties of phosphoribosyl pyrophosphate synthetase from Salmonella typhimurium. J. Biol. Chem. 250: 7492-7500, 1975.

Orenstein, J.M., Shelton, E., and Lazzarini, R.: The association of ribosomes with intracellular vesicular stomatitis virus particles. J. Virol. 16: 447-452, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 945-03 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Study of the Factors Regulating the Synthesis of Collagen in normal and transformed cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Peterkofsky	Research Chemist	LB NCI
OTHER:	R. Hata	Visiting Fellow	LB NCI
	D. K. Kalwinsky	Research Associate	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Biosynthesis Section

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesdda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	2

SUMMARY OF WORK (200 words or less - underline keywords)

Biomedical techniques have been used to determine the submicrosomal localization of prolyl and lysyl hydroxylases, enzymes which catalyze the hydroxylation of the indicated amino acid residues in nascent procollagen chains. While 90-93% of prolyl hydroxylase was found to be intracisternal, only half of lysyl hydroxylase was. The other half was tightly bound to the microsomal membrane and required 0.1M NaCl plus detergent for release. The functional significance of such different distributions for two very similar enzymes is being investigated. Studies also have continued on the identification of a reducing cofactor for prolyl hydroxylase in Kirsten virus transformed Balb 3T3 cells (Ki-3T3). Ascorbic acid is not synthesized by these cells although proline hydroxylation is ascorbate-independent, but they do contain reduced pteridine which can act as a reductant for prolyl hydroxylase in vitro. There is approximately 7-fold more pteridine in transformed cells than in the parent which is ascorbate dependent, suggesting that a pteridine may be the active cofactor in Ki-3T3. Ki-3T3 has a relative rate of collagen synthesis about one-third that of the parent line and synthesizes a different genetic type of collagen than the parent, suggesting that viral transformation alters the genetic expression of collagen synthesis. Dibutyryl cAMP increases collagen synthesis in Ki-3T3 but does not cause reversion to the genetic pattern of the parent.

Project Description:

Objectives: To elucidate the basic mechanisms of collagen synthesis and secretion and to investigate the factors which regulate these processes in cultured fibroblasts, both normal and transformed, and during embryonic development.

Methods Employed:

1. Proteins of whole chick embryos, isolated bones of these embryos, or cell-free systems derived from these bones or cultured fibroblasts are labeled with ^{14}C-proline. The proteins are precipitated with trichloroacetic acid, redissolved and then assayed for radioactivity in collagen and non-collagenous proteins by a method involving specific digestion of collagen in a mixture of proteins using highly purified collagenase. This method was developed in our laboratory and is described in a previous report (1968-9). The relative rate of collagen synthesis can be calculated from data obtained by this method and the specific rate of synthesis in tissue or cells is calculated by determining the DNA content and expressing the rate as cpm/μg DNA. The proteins of the cell and medium fractions of cultured fibroblasts are analyzed separately in order to study secretion. Morphological changes in cultured cells are recorded by polaroid photomicroscopy.

2. Cell-free incorporation of ^{14}C-proline is studied with a system consisting of membrane-bound or free polysomes derived from chick embryo bone tissue and soluble factors derived from chick embryo liver, a tissue which synthesizes very little collagen, so that variations in collagen synthesizing polysomes of a tissue may be studied independently of possible variations in the soluble fraction.

3. To determine the level of proline hydroxylation in collagen of cells or tissues labeled with radioactive proline, collagenase digests are hydrolyzed with 6N HCl and radioactive proline and hydroxyproline in the hydrolyzates are measured by a specific radioassay. To measure the level of lysine hydroxylation, cells or tissues are labeled with radioactive lysine and hydrolyzed collagenase digests are chromatographed on Dowex-50 in 2N HCl, which separates lysine and hydroxylysine.

4. Prolyl and lysyl hydroxylases are measured by tritium release from ^{3}H-proline or ^{3}H-lysine labeled unhydroxylated collagen prepared by incubating chick embryo frontal bones with the labeled amino acid in the presence of the iron chelator α,α^{1} -dipyridyl, which inhibits hydroxylation.

5. Pteridines in cell extracts were measured using purified phenylalanine hydroxylase which has a specific requirement for tetrahydropterins.

6. Collagen types synthesized in cultured cells were determined by analysis of ^{14}C-or ^{3}H-proline labeled, denatured collagen using carboxymethyl cel-

lulose chromatography and gel electrophoresis in sodium dodecyl sulfate-agarose gels. The presence of disulfide bonds was detected by running samples with and without dithiothreitol and observing alterations in the positions of α chains.

Cell Lines used in these studies: Chick embryo fibroblasts: prepared by digesting frontal bones from 15 day chick embryos with crude collagenase and culturing the released cells. Subcultures in the second to fifth passage are used.

L-929- a line of mouse embryo fibroblasts established about 30 years ago by use of a chemical carcinogen.

Balb-3T3- a contact inhibited line of mouse embryo fibroblasts isolated by Todaro and Aaronson.

Balb-3T6 and 3T12- lines of mouse embryo fibroblasts derived from the same pool of cells as 3T3 but non-contact inhibited and tumorigenic.

Ki-3T3- a line established by Aaronson by transformation of Balb 3T3 cells with Kirsten sarcoma virus. These cells do not produce viral particles unless super-infected with murine leukemia virus.

Embryonic and adult human diploid fibroblasts-purchased commercially.

Major Findings:

I. Submicrosomal localization of prolyl and lysyl hydroxylases.

A. Prolyl hydroxylase

Previous work showed that the major portion of prolyl hydroxylase was found in the lumen of isolated microsomes, which would correspond to the cisternae of the rough endoplasmic reticulum. When partial protelolysis with trypsin was carried out, however, a fraction of the prolyl hydroxylase activity was lost, suggesting that at least part of the enzyme might be located on the outer surface of the microsomal membrane. Further experiments have been carried out to clarify this point and the results indicate that almost all of prolyl hydroxylase is intracisternal but that a fraction of the microsomes are slightly damaged so that trypsin and the unhydroxylated collagen chains used as substrate can enter the lumen but the enzyme cannot leak out. In addition, it was found that the molecular weight of prolyl hydroxylase released from microsomes by detergent is approximately twice that reported by several groups for purified chick embryo prolyl hydroxylase. Our results suggest that the enzyme functions as a biomolecular complex or complexed with substrate or another protein.

B. Lysyl hydroxylase

Methods similar to those used to localize microsomal prolyl hydroxylase (see previous annual report) were applied to lysyl hydroxylase. In this case, only 50-60% of the enzyme was released at 0.05% of Triton X-100 or Brij -35, a concentration which releases intracisternal proteins, and even 1.2% Triton would not release any more of the enzyme. Addition of 0.1 M NaCl to 0.4% Triton did result in about 80% release, suggesting that about half of lysyl hydroxylase is very tightly bound to the microsomal membrane and can only be dissociated by a combination of detergent and high ionic strength.

II. Role of ascorbic acid in collagen metabolism.

A. Identity of a reducing cofactor for prolyl hydroxylase in transformed Balb 3T3 cells.

Kirsten sarcoma virus transformed 3T3 (Ki-3T3) shows almost no requirement for ascorbate for collagen secretion or proline hydroxylation, while the parent 3T3 line does. Ki-3T3 does not synthesize ascorbic acid nor is ascorbate present in the culture medium, therefore we have postulated that these cells synthesize a compound which can replace ascorbate as a reducing cofactor for prolyl hydroxylase. Extracts of Ki-3T3 cells were passed through Bio-Gel P-10 columns to remove small molecules which might act as a cofactor. The protein fraction contained prolyl hydroxylase activity which required either ascorbic acid or another reducing compound. Other compounds which were active included tetrahydrofolic acid, tetrahydrobiopterin, tetrahydrodimethyl or 6-methyl pterin and cysteine at about 12-15% the rate with saturating ascorbate, and NADPH and NADH at about 3% the rate with ascorbate; glutathione and coenzyme A had almost no activity. Ki-3T3 cell extracts were examined for pteridine content using an assay with purified phenylalanine hydroxylase which has a specific requirement for 6-or 6,7-substituted pterins. Pterin was present in Ki-3T3 cell extract at about 7 times the concentration in 3T3 extract, suggesting that pterin might be the active cofactor in Ki-3T3 cells. When Ki-3T3 cells were grown in the presence of aminopterin, however, there was no inhibition of collagen secretion, which is a function of proline hydroxylation. This experiment would suggest that a pteridine derivative is not the reducing cofactor.

III.Effect of dbcAMP on transformed 3T3 cells.

A. Genetic expression of collagen synthesis in Balb 3T3, Ki-3T3 and dbcAMP treated Ki-3T3.

Four distinct genetic types of collagen are known at present. Type I, found in almost all tissues but uniquely in calcified bone and tendon, is composed of 2-α_1 type I chains and 1-α_2 chain: $[\alpha_1(I)]_2\alpha_2$. Type II collagen, $[\alpha_1(II)]_3$, is found in cartilage; type III, $[\alpha_1(III)]_3$, mainly in embryonic

tissue and adult blood vessel walls and distinguished from other collagen types by having intrahelical S-S bonds; and type IV in basement membrane. Balb 3T3 was found to synthesize mainly Type I collagen but the α_1/α_2 ratio exceeded 2, suggesting that a small amount of another type composed of $[\alpha_1(X)]_3$ was also synthesized. By contrast, Ki-3T3 synthesized type I and type III, so that transformation appears to have induced expression of a new collagen gene. Treatment of Ki-3T3 cells with dbcAMP increased collagen synthesis 2-3 fold, as previously reported by us, but types I and III remained the predominant types synthesized although a small amount of type X may be induced. These results indicate that increasing cAMP levels in transformed cells does not cause reversion to the nontransformed genotype although collagen synthesis is increased.

Proposed Course of Research

I. Prolyl and lysyl hydroxylase in chick embryo limb bone.

A. Prolyl hydroxylase

The properties of microsomal prolyl hydroxylase will be investigated since preliminary results suggest that the enzyme may function as a large complex.

B. Lysyl hydroxylase

Further experiments will be carried out to determine the exact location of approximately half of the enzyme which is not intracisternal.

II. Role of ascorbate in collagen metabolism.

A. Identity of the reducing cofactor in transformed cells.

Conditions for obtaining Ki-3T3 cell extracts with reducing cofactor activity will be improved and identification will be attempted both by chemical isolation procedures and by studying the properties of the cofactor in the prolyl hydroxylase assay system.

B. Mechanism of action of ascorbate as Vitamin C.

Experiments will be resumed to determine what effect ascorbate has on collagen synthesis and degradation in cultured chick ebryo fibroblasts.

III. Effect of dbcAMP on transformed cells.

Balb 3T3 cells transformed by other sarcoma viruses, which are RNA viruses, and by a DNA virus, SV-40, will be examined to determine whether the alteration in genetic expression of collagen observed in Ki-3T3 cells is a general result of transformation.

Significance to Biomedical Research and the Program of the Institute: Determining the precise mechanism by which vitamin C affects collagen synthesis should lead to a more accurate use of this vitamin. In addition, our studies with transformed cells indicate that, at least under some conditions, animal cells may be able to synthesize a substitute for the function of vitamin C in connective tissue.

Our studies with dbcAMP are aimed at determining the extent to which this chemical reverses cell transformation.

Publications:

Blanck, T.J.J. and Peterkofsky, B.: The stimulation of collagen secretion by ascorbate as a result of increased hydroxylation in chick embryo fibroblasts. Arch. Biochem. Biophys. 171, 259-267 (1975).

Evans, C. and Peterkofsky, B.: Ascorbate-independent proline hydroxylation resulting from viral transformation of Balb 3T3 cells and unaffected by dibutyryl cAMP treatment. J. Cell. Physiol. (In press), 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 5214-05 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

DNA Synthesis in mammalian cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Wilson	Medical Officer	LB NCI
OTHER:	A. Matsukage	Visiting Associate	LB NCI
	M. Sivarajan	Visiting Fellow	LB NCI
	J. Pitha	Research Chemist	LMC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Biosynthesis

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 3/4	1 3/4	1

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha class DNA polymerases from mouse myeloma have been partially purified and examined with regard to physical properties and template-primer specificity.

Project Description:

Objective: The objective of this research program is to understand mechanisms involved in DNA synthesis in mammalian cells. Our approach is the examination of purified DNA polymerases in in vitro systems capable of DNA replication.

Our previous analysis of the DNA polymerases of mouse myeloma revealed several distinct forms of enzyme that were analogous to DNA polymerases well known in other mammalian tissues. These enzymes have now been partially purified, and are being characterized with regard to their molecular properties and mechanisms of action.

We anticipate that our studies on the properties and specificities of the purified DNA polymerases will help answer important question about mechanisms of DNA synthesis in vivo.

Methods Employed: Procedures have been developed for purification of DNA polymerases from mouse tissue. These procedures involve subcellular fractionation, various types of column chromatography and assays for DNA polymerase activities using a variety of reaction conditions. Cell fractionation procedures and characterizations of reaction products were performed using conventional techniques of rate-zonal centrifugation and scintillation spectrophotometry.

Major Findings:

1. Molecular heterogeneity of alpha class DNA polymerase: Alpha class DNA polymerase activity in extracts from MOPC-104E is not associated with a single protein molecule, but with several molecular species that differ in isoelectric point. The three most abundant of these enzyme species were first separated from other DNA polymerases, and then resolved from each other by repeated chromatography on DEAE-cellulose columns. Next, using a new method that involves DNA-cellulose column chromatography, the three species were further purified to approximately 10-20% homogeneity, as judged by polyacrylamide gel electrophoresis under nondissociating conditions. These three highly purified enzyme species exhibited very similar catalytic properties; the main activity of all three sedimented at the the same rate (6-7S) in glycerol gradients containing 0.5M KCl. Analysis of the polypeptide content of two of the species, after further purification by gel electrophoresis, indicated that both were multicomponent; each contained 120,000 dalton polypeptides, but the sizes of the other polypeptides in the two species were different.

We believe that the different species of alpha class DNA polymerase could have been the result of different extents of proteolytic cleavage of the same enzyme molecule. No evidence was obtained that such cleavage occurred in vitro since multiple enzyme species were observed with extracts

prepared under several different types of conditions, including the use of the protease inhibitors, phenylmethanylsulfonyl flouride or trasylol; the species were recovered in about the same relative amounts from both the nuclear and cytoplasmic fractions of MOPC-104E, and multiple enzyme species also were observed in extracts from fetal bovine liver.

2. Determination of the template-primer specificity of a highly purified mouse myeloma alpha class DNA polymerase: The template-primer specificity was systematically examined by measuring initial rates of deoxynucleotide incorporation in various reactions containing combinations of long and short DNA and RNA homopolymer templates and primers. In a test for substrate and product degrading activities, the enzyme preparation was found to be free of levels that could significantly alter the results. The highest activity observed was with poly(dT) as template and oligo(rA) as primer. This combination was 2 to 4-fold more effective than either maximally activated calf thymus DNA or poly(dC).poly(dI), and was at least 10-fold more active than any of the other combinations tested. The enzyme was only slightly active with poly(dI) as template, and with all of the homopolymer templates, changing the sugar from deoxyribose to ribose resulted in a marked decrease in activity. Thus, with regard to use of templates, poly(dT) > poly(dC) $\geq$ Act. DNA >> poly(dA) > poly(dI) = poly(rA) > poly(rC) > poly(rU) = poly(rI), indicating that polydeoxypyrimidine templates were preferred. This specificity was also observed when the various primers and template-primer combinations were compared over a range of concentrations. A high degree of primer specificity was observed with the three well used homopolymer DNA templates and activity was not always proportional to the number of primer termini; for example, 40 µg/ml of oligo(dT) and poly(dT) were almost equally effective in priming poly(dA) replication. Despite the high activity in oligo(rA)-primed poly(dT) replication, the enzyme was relatively inactive in reactions containing oligo(dA) as the primer; relatively low activity was observed with all of the homopolymer RNA primers except oligo(rA). A high degree of primer specificity was not detected with calf thymus DNA as template, since its replication was effectively primed by either DNA or RNA, and all four ribonucleotides were present at the RNA-DNA joints in the product made with the RNA primer.

3. Observations on the mechanisms of action of the myeloma alpha class DNA polymerase: As revealed by competition experiments, a feature contributing to the template-primer specificity appeared to be high binding affinity between the enzyme and the template-primer complex. However, avid binding alone was not sufficient for high activity, since several of the poorly used template-primer combinations were effective in binding the enzyme. In order to examine separately the processes of nascent chain elongation and initiation on a pre-existing primer, replication of single stranded DNA was measured after a template-primer complex was attached to its 3'terminus. The poly(dT).oligo(rA) hybrid initiated replication of denatured calf thymus DNA, whereas, the other combinations of 3'terminal deoxyhomopolymer tract and ribohomopolymer primer did not. Initiation on a template-primer complex

appeared to be sufficient for high enzyme activity, since poly(dI) containing a 3'terminal (dT) tract oligo(rA) hybrid was well replicated. This suggested that the overall template-primer preference reflected the ability of the enzyme to initiate on a template-primer complex. Under a variety of reaction conditions including a large excess of primer molecules, the DNA reaction products were synthesized in the form of short chains, with an average length of about 25 mononucleotides. This suggests that the action of the enzyme was self-limiting with regard to the chain elongation, and that the enzyme was highly processive in its completion of each nascent oligonucleotide chain before initiating a new chain in a distributive fashion.

4. A new method for distinguishing viral and cellular DNA polymerase using the (dT)·(rA) hybrid as template-primer: We have found that mouse enzymes representative of three classes of cellular DNA polymerase, alpha, beta and gamma, efficiently use poly(dT)·oligo(rA) as a template-primer for the synthesis of poly(dA). In contrast, the RNA virus particle associated DNA polymerases are virtually inactive with poly(dT).oligo(rA). This difference provides a simple new way of distinguishing these cellular and RNA virus particle associated DNA polymerases.

5. A template specific inhibition of DNA polymerase activity: We have found that poly(9-vinyladenine), is a template specific inhibitor of mammalian DNA polymerases; the polymer selectively blocks the replication of DNA containing poly(dT) sequences. Thus, poly(dT)-directed poly(dA) synthesis by representatives of all three classes of cellular DNA polymerase could be completely inhibited by poly(9-vinyladenine), although higher concentrations were required in the case of the gamma class enzyme. Studies on the mechanism of the inhibition using the alpha class DNA polymerase and different templates showed that the enzyme activity was inhibited in all cases where base-pairing between the vinyl polymer and the template occurred; poly(9-vinyladenine) did not interfere with the replication of templates to which it does not bind. The inhibition occurred shortly after addition of poly(9-vinyladenine) to ongoing reactions, yet the enzyme was not displaced from the template - primer complex.

Significance to Cancer Research: Detail knowledge of the mechanisms involved in DNA synthesis is vital to our understanding the molecular biology of neoplasia. Many approaches are being used to investigate DNA synthesis in normal and neoplastic cells, and advances on all levels will prove useful in preventing, treating, and ultimately controlling cancer. Activity of DNA synthesizing enzymes is necessary for maintenance of a rapid rate of cell division, and in some cases these enzymes may play an important role in the development of the neoplastic state.

Proposed Course of Research: The objective of this research program is to understand mechanisms of DNA synthesis in mammalian cells; the main approach is the investigation of the mechanism of action DNA synthesizing enzymes *in vitro*..

1. To purify the alpha and gamma class DNA polymerases of mouse myeloma.

2. To investigate the mechanism of action of purified alpha class DNA polymerase using the prototype system of poly(dT) as template and oligo(rA) as primer.

Publications:

Matsukage, A., Bohn, E.W., Wilson, S.H.: A Method for Distinguishing RNA Virus Particle and Cellular DNA Polymerase Acitivities Using the (dT) (rA) Hybrid as a Template-Primer. Nuc. Acids Res. (In press).

Pitha, J., Wilson, S.H.: Template Specific Inhibitor of Mammalian DNA Polymerases. Nuc. Acids Res. (In press).

Minna, J.D., Marshall, T.H., Burk, R.D., Lemons, R.S., Brown, S.H., Wilson, S.H.: On the Somatic Cell Genetics of Type C Viral Particles: Regulation of Virion DNA Polymerase in Human X Rodent Hybrid Cells. Proc. Nat. Acad. Sci. USA (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 5215-05 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological and ultrastructural studies on cell cooperation in the immune response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Emma Shelton	Research Biologist	LB NCI
OTHER:	Jan M. Orenstein	Senior Surgeon	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Biosynthesis Section

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

To examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen

To continue with scanning electron microscopy to:
Characterize surface morphology of lymphocytes and macrophages derived from the peritoneal cavity. Compare normal and immune cells in presence and absence of antigen. To describe surface morphology of cells that have differentiated into plasma cells in diffusion chambers.

To prepare hybrid antibody to tag T and B cells for identification in SEM and to follow fate of these cells in differentiation in diffusion chambers and in the milk spots of the omentum.

Project Description:

Objectives: To examine the interaction of lymphocytes and macrophages co-operating in the immune response and, specifically; to examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen.

Methods and Major Findings: Criteria for the "normal" appearance of the surface of lymphocytes, macrophages, polymorphonuclear leukocytes and mast cells. This was done by comparing the surface of cells fixed in situ in the peritoneal cavity with similar cells that had settled on artificial substrates. The mast cell surface is similar in both environments while the lymphocyte surface is more pleomorphic in vivo. The macrophage may flatten on an artificial substrate to the extent that it loses all surface convolutions. In vivo, the flattened macrophage always retains a folded, rippled, convoluted surface.

In the course of studying the individual cells in situ with scanning electron microscopy, some anatomical features of the mesenteries, omentum and diaphragm were elucidated. These surfaces are patent and permit the passage of free cells through lacunae into the underlying lymphatic vessels.

We are attempting to find factors that regulate the structure of the surface of the lymphocyte and have exposed peritoneal cells to the following metabolic inhibitors: --KCN, NaN_3, iodoacetamide, NaF, dinitrophenol, puromycin and EDTA. Of these only iodoacetamide (5×10^{-3} M) had any effect upon surface morphology after one hour exposure. In the presence of iodoacetamide, both lymphocytes and macrophages lost microvilli and became covered with deeply scalloped surface folds. It was also found that exposure of these cells to temperatures of 0°-4° C caused them to become covered with long, spike-like microvilli. This cold effect was not abrogated by iodoacetamide.

Membranes of flattened macrophages engulf sheep erythrocytes (SRBC) differently from membranes of spherical macrophages. The latter arise from several sites at the point of contact with the SRBC and cover it in overlapping folds somewhat like the fins of an iris diaphragm while the former create a tube-like structure in which the SRBC is engulfed. We hypothesize that the convoluted surface of the spherical macrophage permits initiation of multiple flaps of membranes, while a single contact with the smooth surface of the flat macrophage stimulates the formation of a single, funnel shaped membrane.

Proposed Course: To examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen and, specifically; to continue with scanning electron microscopy to characterize and compare surface morphology of lymphocytes

derived from the peritoneal cavity, thymus and lymphnode. To describe surface morphology of cells that have differentiated into plasma cells in diffusion chambers.

To prepare hybrid antibody to tag T and B cells for identification in SEM and to follow fate of these cells in differentiation in diffusion chambers.

Publications:

Schneider, W.C., Shelton, E., and Kuff, E.L.: Association of DNA with melanin granules. J. Natl. Cancer Inst. 55: 665-670, 1975.

Shelton, E. and Orenstein, J.M.: A plating method for preparation of cells for culture and for observation by light or electron microscopy. Exptl. Mol. Path. 23: 220-227, 1975.

Shelton, E.: Scanning biological organization. Cell 5: 340, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5241-01 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Transcription of covalently linked cellular and SV40 viral DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Kuff	Head, Biosynthesis Section	LB NCI
OTHER:	G. Khoury	Head, Section on Virus Tumor Biology	LDTV NCI
	E. Winocour	Professor of Virology	Not NIH

COOPERATING UNITS (if any)

Viral Biology Branch, NCI
Weizmann Institute of Science, Israel

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Biosynthesis Section

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

We have begun to examine the transcription of covalently linked cellular and viral DNA sequences during infection with host substituted variants of SV40. Procedures have been developed for detecting transcription of specific host sequences contained in defective substituted viruses.

Project Description:

Objectives: SV40 DNA becomes covalently integrated into the host cell genome during transformation of nonpermissive cells and probably in the course of lytic infection. This association may have important effects in regulating expression of both host and viral genetic information; however, direct studies of such effects are technically difficult because the virus is usually integrated in small numbers. Another approach is afforded by the use of defective substituted SV40 variants, in which parts of the closed circular viral DNA have been deleted and replaced with covalently linked host cell DNA. In mixed infections with wild type virus as helper, these variants can replicate and reach large numbers in the nuclei of affected cells. We are examining the transcription of host and viral sequences during infection of monkey cells with such host substituted SV40 virus.

Methods Employed: Cell culture; virus passage; nucleic acid labelling, isolation and hybridization; other biochemical and molecular biological techniques.

Results: Procedures have been developed for measuring the transcription of specific host sequences contained in defective substituted viruses. Following infection with one well-characterized variant, we were unable to detect transcripts of the host sequences in the total RNA from chronically labelled cells or in nuclear RNA after 3 hours of labelling. However, when viral "transcription complexes" were isolated from the nuclei of infected cells and incubated *in vitro* with labelled nucleotide triphosphates, host sequences were found to be transcribed. It appears, therefore, that transcripts of this particular substituted variant have some property which results in their rapid turnover in the intact cell.

Proposed Course of Research: Transcription and post-transcriptional events will be studied in detail following infection with the above and several other substituted SV40 variants.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3212-02 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on cell nutrition, growth and neoplastic transformation in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. M. Price	Research Biologist	LB NCI
OTHER:	K. K. Sanford	Head, Research Biologist	LB NCI
	C. H. Fox	Scientist	LB NCI
	C. Garrison	Technician	LB NCI

COOPERATING UNITS (if any) R. Parshad, Dept. of Pathology, Howard Univ., Wash., D.C. K. Kohn, Lab. of Molecular Pharmacology, NCI; J. Selkirk, Chemistry Branch, NCI; R. Merwin, Viral Leukemia and Lymphoma Branch, NCI; R. Deeley, Cellular Regulation Section LB, NCI.

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cell Physiology and Oncogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5 technical

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the in vitro nutritional requirements of freshly isolated cells and long-term lines, both neoplastic and nonneoplastic and to improve culture conditions of the cells so that rigidly controlled cells can be used to study the differences between nonneoplastic neoplastic or malignant cells at nutritional, metabolic, karyologic and morphologic levels.

Project Description: A program to improve culture conditions so that rigidly controlled cells can be used to study differences between nonneoplastic and neoplastic cells at nutritional, metabolic, karyologic and morphologic levels.

Objectives:

1. To determine the effect of horse and fetal bovine serum and 1% and 18% oxygen on chromosome stability, presence of fetal antigen and DNA breakage of C57Bl mouse lung cells.

2. To determine if a more sensitive in vivo assay for neoplastic transformation can be obtained by implanting glass helices carrying mouse cells into syngeneic hosts.

3. To develop techniques for culturing normal human epithelial cells and to attempt to transform these cells by chemical carcinogens and study differences between normal and neoplastic human epithelial cells at nutritional, metabolic, karyologic and morphologic levels.

4. To develop methods for culturing chick liver cells in vitro. Once in culture those cells will be used to study regulation of certain metaboloic functions of chick liver.

Methods Employed: Cell cultures derived from adult C57Bl mouse lung were cultured in medium containing either horse serum or fetal bovine serum and cultures grown in both sera were gassed with 1% and 18% oxygen-containing gas. The cells were tested at various intervals for presence of fetal antigen, changes in chromosomes and DNA breakage and for neoplastic transformation.

Techniques were devised to culture normal human epithelial cells. Primary cultures of human epithelial cells were treated with various chemical carcinogens to attempt to induce neoplastic transformation.

Methods were developed for culturing chick liver cells in vitro.

Major Findings:

1. The effect of horse and fetal bovine serum and 1% and 18% oxygen on chromosome stability, DNA breakage, production of fetal antigen and neoplastic transformation in C57Bl mouse lung cultures. The chromosome number of C57Bl mouse lung cells remained more stable when the cells were grown in an atmosphere containing 1% oxygen than when the cells were cultured in the presence of 18% oxygen. This effect was noted regardless of the serum which was in the culture medium.

Tests are still being run to determine the effect of horse and fetal bovine serum and 1% and 18% oxygen on DNA breakage and repair of the lung cells.

All of the cell lines tested showed no presence of fetal antigen through 10 weeks in vitro. After approximately 20 weeks in culture the cells grown in fetal bovine serum and 1% oxygen showed some fetal antigen. After 40 weeks in vitro the cells in horse serum and 1% oxygen showed fetal antigen as well as those cells in fetal bovine serum in either oxygen concentrations. There appeared to be more fetal antigen present, however in the cells grown in fetal bovine serum in 1% oxygen.

All cell lines tested became neoplastic by in vivo assays. The latent period for cells grown in medium containing horse serum was shorter (2 months) than for all other lines tested (from 6 to 8 months).

2. Development of a more sensitive assay system for neoplastic transformation. C57B1 mouse cells were grown on the surface of 3mm glass helices placed in a Yoshi culture dish. Under the direction of Dr. Ruth Merwin a small number of the helices carrying cells were implanted subcutaneously into syngeneic hosts. From 3 to 6 helices were placed in each animal. Each helix contained approximately 100 cells. Tumors were obtained in 3 of 4 cell lines injected indicating that it is not necessary to have very large numbers of cells. The latent period for the tumors was slightly longer than for tumors derived by the usual method of innoculating at least 10^6 cells intramuscularly.

3. Development of techniques for the cultivation of human epithelial cells and treatment of those cells with chemical carcinogens.

Human epithelial cultures are obtained by placing 1-2 mm explants into culture flasks coated with serum. Within 7-10 days epithelial growth is obtained and the explants are removed. Persistant fibroblasts are gotten rid of by frequently trypsinizing the culture. The epithelial cells remain attached. All attempts at subculturing the epithelial cells have failed. We are now in the process of trying to grow the epithelial cells on X-irradiated feeder layers of fibroblasts in the hope of attaining better growth and perhaps the ability to subculture the cells.

Attempts to neplastically transform epithelial cells with benzpyrene have been unsuccessful although we have determined that human epithelial cells metabolize this carcinogen. Results of these studies are given in the report of Cecil Fox, Serial No. 5239-01 LB.

4. Development of methods for the cultivation of chick liver cells.

Initial attempts at culturing chick liver cells were made with young roosters 3 to 9 weeks old. We tried several media including NCTC 135 and NCTC 163 and Leibovitz L.15. The NCTC 163 and L.15 both contain large amounts of amino acids which we thought would be helpful. It appears thus far that better growth is obtained with NCTC 135. Fetal bovine serum is used as a supplement. We have found that more success is obtained by dispersing the liver tissue with pronase than by just mincing and planting liver explants. We are presently attempting to establish long-term chick liver cell lines.

Significance and Proposed Course:

1. Data from this project is being analyzed. We hope to be able to correlate changes in chromosomes, DNA and fetal antigen with certain culture conditions and with neoplastic transformation. Continued work in this area is described in the report of Dr. K. Sanford, Serial No. 3213-01 LB.

2. This was a short-term project to determine whether methods requiring fewer cells could be used for in vivo assay for neoplastic transformation. This may be useful for assaying cell lines which do not proliferate rapidly or which do not readily become established long-term lines such as human epithelial cultures.

3. Most human cancers are carcinomas. It would be beneficial if in vitro studies can be carried out with epithelial cells rather than fibroblasts. Traditionally however most in vitro cancer studies have used fibroblasts since they are much easier to cultivate. It is vitally necessary to develop techniques allowing investigators to culture and study human epithelial cells. We will continue to improve methods for the cultivation of these cells. We will use these cells to further study neoplastic transformation by carcinogenic agents. We are also studying morphology of carcinogen-treated epithelial cells by exfolliative techniques commonly used by pathologists.

4. We are still trying to obtain readily growing cultures of chick liver cells. The cultures once obtained will be used to study metabolic functions of the liver cells.

Publications:

1. Fox, C. H., Selkirk, J.K., Price, F.M., Croy, R.G., Sanford, K.K. and Cottler-Fox, M.: Metabolism of benzo(a)pyrene by human epithelial cells in vitro. Cancer Res. 35: 351-357, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3213-02 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiology and Oncogenesis of Mammalian Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. K. Sanford	Head, Cell Physiology & Oncogenesis	LB NCI
OTHER:	S. Handleman	Biologist	LB NCI
	G. Jones	Microbiologist	LB NCI
	R. Tucker	Medical Director	LB NCI

COOPERATING UNITS (if any) R. Parshad, Dept. of Pathology, Howard Univ. School of Medicine, Wash., D.C., K. Kohn, Lab. of Molecular Pharmacology, B. Wetzel, Dermatology Branch, NCI, B. Bower, Laboratory of Cell Biology, HI

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of the following studies: Study 1. (Collaboration with Dr. Parshad). The effect of culture conditions on karyotypic stability of rodent and human cells in culture.

Objective: To define those culture conditions that induce or prevent chromosomal instability in cultured cells, to analyze the mechanisms, and to evaluate this type of genetic change as a causative factor in "spontaneous" neoplastic transformation of rodent cells in culture.

Major Findings:

Decreasing the oxygen concentration in the gas phase from atmospheric (18%) to 1% decreased the frequency of chromosomal aberrations in mass cultures of both adult and embryo mouse cells. Both the rate of shift from the diploid number and the incidence of abnormal chromosomes were decreased at the lower concentration. Exposure of mouse cells to room lights (cool white, fluorescent) during routine fluid renewals increased the incidence of abnormal chromosomes, particularly minutes and metacentrics. These abnormalities originate from breaks in chromatin fibers during interphase. From known effects of light and oxygen in photosensitizing riboflavin to produce oxidative changes in amino acids, proteins, and DNA, we postulate that the increased frequency of chromatin breaks results from a photodynamic reaction affecting the DNA or associated proteins of the chromatin fiber.

Proposed Course: The cytostatic and inhibitory effects of light and oxygen on cells will be explored particularly with varying amounts of riboflavin in the medium. The effect of light exposure on DNA strand breakages will be tested in an effort to obtain a rapid assay for factors in the culture medium which may enhance or prevent chromatin fiber breakage and specifically DNA breakage or linkage of strands to each other or to protein (in collaboration with Dr. K. Kohn). An effort will be made to stabilize chromosomes and determine if such stabilization prevents spontaneous neoplastic transformation or conversely to induce chromosome changes by varying light exposure, oxygen, and medium components such as riboflavin and tryptophan.

Significance: Elucidation of the causative factors and mechanism of chromatin strand breakage in rodent cells, which have an impaired DNA excision repair mechanism, may have considerable practical and theoretical significance for understanding and controlling genetic and possibly spontaneous neoplastic change in cultured cells.

Study 2. The surface topography, plasma membranes, and cell coat of paired neoplastic and nonneoplastic cells in culture. (Completion of studies reported previously and initiation of a new study).

Objectives: 1. To compare the surface features, (microvilli, blebs, and ruffles) and form of paired neoplastic and nonneoplastic cells to establish whether the less spread neoplastic cell has excessive amounts of membrane stored in cell projections and thus appears to have a greater surface activity. (Revised manuscript ready).

2. To establish whether spontaneously transformed neoplastic cells have a thicker acid mucopolysaccharide coat and adherent serum proteins (manuscript in press).

3. To examine the pattern of protein distribution in cell membranes of nonneoplastic and neoplastic cells by freeze-etch EM (collaboration with Dr. B. Bowers).

Major Findings:

Cells of neoplastic lines were characteristicly less spread and displayed greater variability in surface features. However, they were not more villous and the incidence of microvilli, ruffles and blebs did not prove reliable criteria of neoplastic state in SEM. Ruthenium red staining which visualizes both acid mucopolysaccharides of the cell coat and adhering serum components indicated similar thicknesses in neoplastic and nonneoplastic cells of common origin. Although a thickening of coat has been reported in some virus-transformed cells, such a differential thickening was absent from these spontaneously transformed cells.

Proposed Course:

The pattern of protein distribution in the plasma membrane will be examined since a disoriented pattern has been reported to characterize certain carcinoma cells and may be a surface alteration recognized by activated macrophages.

Study 3. Locomotor behavior of nonneoplastic and neoplastic cells in culture.

Objective: To analyze and explain differences in locomotive pattern in nonneoplastic and neoplastic cells of common origin.

Major Findings:

In film analyses to date, the neoplastic cells exhibit a greater randomness of direction in locomotion than nonneoplastic counterparts under the same culture conditions and regardless of cell density or total period of life in culture. These observations probably explain the colony patterns diagnostic of so many cell types after neoplastic transformation. Further analyses will be made to establish the generality of this parameter of cell behavior in neoplastic cell populations.

Significance:

This observation concerning randomness of motion indicates a certain loss of polarity in the neoplastic cell. The underlying mechanism may be associated with changes in cell adhesion or in the endoskeleton of the neoplastic as compared with nonneoplastic cell.

Study 4. In vitro correlates of spontaneous neoplastic transformation.

Objectives: 1. To develop and evaluate rapid tests for spontaneously transformed neoplastic cells in culture, and 2. To establish whether spontaneously transformed cells exhibit properties similar to virus-transformed cells.

Major Findings:

Extensive studies of 7 pairs of nonneoplastic and neoplastic cell lines were carried out to establish whether rigorous correlations exist between certain

colony characteristics and migration patterns (now being adapted to quantitation by computerized image analysis), growth in agarose, and tumorigenicity in syngeneic hosts. Good correlations between these two in vitro properties with tumorigenicity were established. However, spontaneous transformants were found to differ from virus transformants in showing very low plating efficiencies in agarose,

Significance:

The establishment of good correlations between spontaneous neoplastic transformation (as assayed by tumorigenicity) and both colony morphology and growth in agarose has considerable practical as well as theoretical significance. The practical advantage is that we now have a rapid means for monitoring spontaneous neoplastic transformation in cell populations in culture. The theoretical significance is that one property, regardless of cell type, seems to underlie neoplastic change i.e., tendency to cell rounding (or reduced cytoplasmic spread) associated with loss of surface-substrate dependence. The studies also show that spontaneous as well as viral transformants show this change but to a lesser degree.

Study 5. Search for Moloney sarcoma (MSV) RNA in spontaneously transformed mouse cell lines. (See last year's report). Completed and terminated.

Study 6. Microfluorimetry studies on metabolism of intact cells (see last year's report). Recently reactivated with the return of Dr. Cohen to his laboratory.

Honors, Awards and other Activities

1. Council member, Tissue Culture Association
2. Member, Editorial Advisory Board of Cancer Research
3. Member, Terminology Committee, Tissue Culture Association
4. Chairman, Earle Award Committee, Tissue Culture Association
5. Member, Program Committee, 3rd Decennial Review Conference, Tissue Culture Association
6. Executive Board, 3rd Decennial Review Conference on "Gene Expression and Regulation in Cultured Cells."

Publications:

1. Fox, C.H., Price, F.M., Sanford, K.K. and Cottler-Fox, M.: Metabolism of Benzo(a)pyrene by human epithelial cells in vitro. Cancer Res. 35: 3551-3557, 1975.

2. Fox, C.H., Dvorak, J.A. and Sanford, K.K.: Cytometric analysis of neoplastic transformation of vertebrate cell populations. Cancer Res. 35: 3551-3557, 1975.

3. Schilling, E.L. and Sanford, K.K.: Preparation of chicken plasma without the use of anticoagulants. Tissue Culture Association Manual, Techniques, Methods and Procedures for Cell Tissue and Organ Culture. I #2, pp. 63-66, 1975.

4. Meltzer, M., Tucker, R.W., Sanford, K.K., and Leonard, E.J.: Interaction of BCG activated macrophages with neoplastic and nonneoplastic cell lines in vitro: Quantitation of the cytotoxic reaction by release of 3_H-thymidine from pre-labeled target cells. J. Natl. Cancer Inst. 54: 1177-1184, 1975.

5. Fox, C.H. and Sanford, K.K.: Chemical analyses of mammalian sera commonly used as supplements for tissue culture media. Handbook of Tissue Culture Techniques. Tissue Culture Association Publisher, Vol. 2, 1976.

6. Sanford, K.K., Hall, W.T., Hobbs, B.A., and Hursey, M.L.: Ruthenium red-staining coat of paired neoplastic and nonneoplastic mouse cell lines in culture. J. Natl. Cancer Inst. (In press) 1976.

7. Taylor, W.G., Evans, V.J., Fox, C.H., Camalier, R.F., and Sanford, K.K.: Evaluation of the dyna-cell vessel for bulk production of substrate-dependent cells, Biotechnology and Bioengineering. (In press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3234A-04 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Primary biochemical alterations leading to neoplastic conversion.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. R. Gantt	Research Chemist, CPO	LB NCI
OTHER:	K. K. Sanford	Head, CPO	LB NCI
	P. Izzo	Visiting Fellow, CPO	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL: 2	OTHER: 2

SUMMARY OF WORK (200 words or less - underline keywords)

This program is designed to elucidate the primary chemical alterations leading to neoplastic transformation. Various biochemical techniques are being applied to a system developed in the Cell Physiology & Oncogenesis Section, with these immediate objectives in view:

1. To identify the primary changes in nucleic acid methylation during neoplastic transformation and cell regulation.

2. To assess the role of nucleic acid methylation in the RNA tumor virus life cycle and its reglationship to transformation.

Project Description: To elucidate the primary chemical alterations which are necessary biochemical changes leading to neoplastic transformation and to devise methods to detect and control the transformation based on these chemical alterations.

Objectives: To identify primary changes in the methylation of cellular and viral nucleic acids during neoplastic transformation and to exploit these changes to develop an assay for, and possible control of, neoplastic transformation in vitro.

Methods Employed: In addition to the usual laboratory techniques and inovations, we use cloned, paired cell lines to control the wide variations among cell lines and even in the same cell line at different culture generations. These paired lines are described by Dr. Katherine Sanford.

Major Findings:

1. A procedure for purifying RNA methylases has been developed which plausibly may be a useful general method. Essentially it consists of two types of affinity columns: One containing S-adenosylhomocysteine as the ligand and the other contains an appropriate nucleic acid as the ligand. Using this combination can be expected to simplify investigation of the numerous generally unstable RNA methylases.

2. Methods have been developed using pressure chromatography to accurately determine 5-methylcytosine in small amounts of DNA without the use of radioisotopes. This technique will give information which may help provide insight into the biological function of eukaryotic DNA methylation.

Significance: The importance of nucleic acid methylation has recently been emphasized by the discovery of post transcriptional mRNA modifications which are required for translation of the message. Work on the biological functions of the numerous specific methylases present in cells has been hindered by lack of specific, sensitive techniques for studying the events. This program is designed to develop the requisite techniques and apply them to the problems of regulation and malignant conversion of cells where nucleic acid methylation has been so often implicated.

Proposed Course of Investigation:

1. Establish whether the N-guanine methylase is essential for the virus life cycle.

2. Examine the distribution of 5-methylcytosine in chromatin fragments derived in various ways to see if the minor base is distributed in a non-random way.

3. Continue to purify selected methylases to prepare antibodies for single cell fluorescent assays and quantitation of specific enzymes on small numbers of cells.

4. Examine the controversial question: Does mitochondrial DNA contain 5-methylcytosine?

Publications:

1. Gantt, R.R., Smith, G.H. and Julian, B.: Virion-associated and cellular RNA methylase activity in normal and neoplastic mammary tissue from MMTV infected and uninfected mice. Cancer Res. 35: 1847-1853, 1975.

2. Gantt, R.R., and Smith, G.H.: RNA methylase activity in virus-infected normal and neoplastic mouse mammary tissue. In "The Biochemistry of S-Adenosylmethionine", edited by E. Borek, F. Salvatore, F. Schlenk, G. Williams-Ashman, and V. Zappia. Columbia University Press, New York, 1976, In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3257-02 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Growth and Neoplastic Transformation of Mammalian Cells In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. G. Taylor	Research Biologist	LB NCI
OTHER:	K. K. Sanford	Research Biologist	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purposes of this program is to design a better synthetic milieu which freshly explanted or long term mammalian cells will survive and grow. Topics of present interest include: (1) the role of oxygen in survival, growth, and metabolism of cells, particularly sparce cell populations; (2) chemical analyses of a presumed-peptide growth factor for mammalian cells grown in serum-free medium.

Project No. 1. Enhanced proliferation at physiologic oxygen concentrations.

Objective: Earlier studies showed colony development (i.e., growth) and chromosome stability are enhanced when a gas phase of 1% rather than 18% oxygen is used in conjunction with 10% CO_2. The partial pressure of dissolved oxygen in medium equilibrated with 1% O_2 is comparable to that of blood and tissues (30-40 mm Hg). Studies were designed to determine if: (a) cell populations obtained with a gas phase of 1% O_2 were "density dependent": and (b) the use of 1% O_2 v/v altered glucose utilization and lactic acid production.

Methods Used: Dissolved gas concentrations and pH were measured with a Radiometer BSM3MK2 blood gas analyzer in conjunction with a PHM 27 meter fitted with a PHA927b gas monitor. Medium glucose concentration was measured with a Beckman glucose analyzer. Lactic acid concentration was measured by a pyridine nucleotide coupled spectrophotometric lactic acid assay. Medium F-12 or Dulbecco-Vogt's modified MEM were used with 10% fetal bovine serum. Cell lines tested included human diploid WI-38 cells and cloned mouse lines NCTC7914 and 7915. Colonies of greater than 0.1 mm diameter were counted, or total cell populations enumerated with a 6300A Cytograf.

Major Findings: (a) An inverse relationship between colony development and Po_2 of the culture medium was observed in the first experiments of this study. Multiple regression analysis of these data confirmed that colony development was significantly effected by the oxygen concentration and initial population size. When, in a similar study, cell numbers (rather than colony counts) were determined, it was clear that with an initial population of 10^4 cells/T-15 or less, 2.5-5 times more cells were obtained with 1% O_2 than in cultures gassed with 18% O_2.
(b) Per culture vessel significantly greater concentrations of glucose are used and lactate produced when 1% O_2 is used. When data are normalized as μmoles/10^4 cells, glucose utilization and lactate production varied inversely with the initial inoculum size and oxygen concentration. A glucolysis ratio of less than 2 was observed in most cases.

Significance and Proposed Course: Whether present as a deliberately introduced CO_2: air mixture or as residual, supernatant gas, a gas phase containing atmospheric oxygen concentrations (18%, medium Po_2 ~125-135 mm Hg.) introduces an essential nutrient at an abnormal concentration. In vivo, inhaled air (Po_2 ~135-150 mm Hg.) equilibrates with alveolar blood (Po_2 ~20-40 mm Hg), and hemaglobulin maintains a homeostatic Po_2 and Pco_2. In vitro, a Po_2 of 30-40 mm Hg can be obtained by equilibrating the growth medium with 1% O_2: 10% CO_2:N_2 v/v in gas-tight, glass culture vessels.

To complete the present study it is important to determine if the enhanced growth at 1% O_2 results from cellular responses to a more physiologic milieu -- that is, does dissolved oxygen regulate cell proliferation. Alternatively, the relatively diminished growth with atmospheric oxygen concentration might be caused by production of cytotoxic products such as hydrogen peroxide

(Science 191: 468, 1976). Further collaborative studies in the role of dissolved oxygen, light and riboflavin (PNAS 71: 3961, 1974) on chromosome instability are planned.

Project No. 2: Further analyses of a peptide-like growth factor for mammalian cells in serum-free medium.

Objectives: Autoclavable, low molecular weight fractions (PD) from commercially available peptones (tissue digests) promote continuous proliferation of mouse, rabbit, hamster, monkey and human cell lines. Studies included: (a) completion or confirmation of earlier experiments regarding the chemical nature of PD; and (b) preliminary purification of PD by chromatographic procedures.

Methods Used: The bioassay techniques used in these studies has been described (Taylor, W.G. and Evans, V.J., Methods In Cell Biology, Vol. VIII, p. 47, 1974). Preliminary chromatographic purification was performed on a 1 x 10 cm Chelex 100 (Na^+ form) column, followed by biologic assay of the effluent material. Assessment of CM-52 and gel filtration procedures (G-10 and G-25, P-2) are in progress. The tripeptide glycylhistidyllysine (Picket and Thaler, Nature New Biology 243: 85, 1973) was tested at 0.1 - 80 μg/ml medium.

Major Findings: Chelex-100 (Na^+) chromatography of PD results in a 1.3-1.4 fold purification, as measured by cell yields obtained in medium containing treated and untreated PD. Glycylhistidyllysine altered cell morphology dramatically, but did not enhance the degree of proliferation in cultures incubated in peptide-containing medium.

Significance and Proposed Course: Preliminary pronase digestion studies suggest a slight decrease in activity, but a well controlled study is needed to determine if PD is sensitive to protease action. More importantly cells adapted to growth in serum-free medium provide a better model for assessing the role of peptide-like hormones on synthesis and mitosis since, in this case cell survival in the absence of serum is certain and no serum-derived protease are present.

Publications:

1. Taylor, W.G., Evans, V.J., Fox, C.H., Camalier, R.F. and Sanford, K.K.: Evaluation of the Dyna-Cell Vessel for Production of Surface-Substrate Dependent Cells. Biotech. & Bioeng. 17: 1847, 1975.

2. Taylor, W.G. and Parshad, R.: Peptones as Serum-Substitutes for Mammalian Cells in Culture. Methods in Cell Biology (D. M. Prescott, Ed.), Vol. 15, In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3263-01 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Bulk Production of Mammalian Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. G. Taylor	Research Biologist	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Cell Physiology and Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0 - Terminated		

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project was to develop Bulk Production Methods for culturing gram amounts of mammalian cells, particularly nonneoplastic and human diploid cells, that require a surface on which to attach and divide. In a new commercially available culture vessel, human diploid fibroblasts grew well provided optimal physiologic conditions were maintained by means of complete medium renewal(s) as indicated by glucose utilization. A mixture of pancreatin-versene was found most successful for recovering Phase II WI-38 cells in excellent physiologic condition.

Project Description: Nonneoplastic and some neoplastic cells require a surface upon which they can attach, migrate, and divide. This study evaluated a new means of growing gram amounts of "substrate-dependent" mammalian cells in a single culture vessel.

Objectives: Efforts were made to: (1) develop a rapid, physiologic means for dispersing adherent cell populations from the growth surface, and (2) determine if a complete change of medium after 24 hours incubation, a high glucose concentration, or the use of insulin would promote more rapid growth of WI-38 human diploid fibroblasts.

Methods Used: The Dyna-Cell^{R} is a cylindrical vessel which houses a spiraled plastic growth surface. Cells and medium (1.6 - 1.7 l) are introduced through ports on top of the vessel, and the culture medium aerated and gently circulated by intermittent gassing with 5% CO_2 in air. Since the inner layers of plastic cannot be visualized, cell growth and the need for medium renewal are assessed indirectly by measurements of available glucose.

Major Findings:

1. Gram quantities of nonneoplastic, human diploid fibroblasts (WI-38) can be grown and recovered from a Dyna-Cell^{R} vessel provided: (a) an inoculum of 5-8x10^{7} cells (~0.5cc, packed cell volume) is used; (b) medium glucose is monitored to assess the need for a complete change of culture medium; and (c) unchilled cell populations are harvested with 0.125% v/v pancreatin-0.01% v/v versene. Cell populations recovered were >95% viable by trypan blue exclusion procedures.

2. With existing medium and methods a 2-5 fold increase in WI-38 cell number is achieved in 7-10 days. A protracted lag in glucose utilization, and presumably proliferation, is observed during the first 72 hours incubation. Neither a complete change of medium at 24 hours (to remove cell debris, residual pancreatin, etc.), the use of an enriched culture medium, nor the use of one IU of insulin per ml medium shortened the growth lag or significantly enhanced cell-yield.

Significance and Proposed Course: A single Dyna-Cell^{R} vessel can produce gram quantities of substrate-dependent cells for tumorigenesis assays, biochemical studies of cell products, membranes, organelles, etc., and will be used to produce large quantities of nonneoplastic cells which can be frozen as a reference stock for future experiments.

Terminated. March, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3264-03 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Spontaneous Neoplastic Change During Growth in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. W. Tucker	Research Associate	LB NCI
OTHER:	K. K. Sanford	Research Biologist	LB NCI
	S. L. Handleman	Biologist	LB NCI
	G. M. Jones	Microbiologist	LB NCI

COOPERATING UNITS (if any) M. S. Meltzer, Biology Branch, NCI
H. Sato, Biophysical Cytology, Biology Dept., Univ. of Pa., Phila., Pa.
F. Frankel, Microbiology Dept., Univ. of Pa., Phila., Pa.
V. Speeg, Lab. of Molecular Biology, NCI

LAB/BRANCH Laboratory of Biochemistry

SECTION Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The neoplastic change in tissue culture is studied by four different approaches.

1. Serum dependence of neoplastic and nonneoplastic cells.
2. Selective susceptibility of neoplastic cells to BCG activated macrophages.
3. The organization of microtubules and microfilaments in neoplastic and nonneoplastic cells.
4. Growth of nonneoplastic and neoplastic cells in agarose.

Methods Employed:

1. The growth of neoplastic and nonneoplastic cells was measured in medium supplemented with different amounts of serum.
2. The cytotoxic reactions of BCG-activated mouse peritoneal macrophages was tested on neoplastic and nonneoplastic cells.
3. The organization of microtubules and microfilaments was measured by the birefringence of these structures in living cells (polarization microscope) and by indirect immunofluorescence in fixed cells.
4. The growth of cells as discrete colonies in agarose was measured in the light microscope.

Major Findings:

1. Neoplastic and nonneoplastic cell descendents from the same cloned single cell have the same growth curves in the same medium. This data has been included in the study on susceptibility to BCG activated macrophages to demonstrate that cell lines from a single cell and with similar growth curves in vitro, react differently in the macrophage assay.

2. a) The susceptibility to mouse BCG-activated macrophages of long term neoplastic cell lines appeared to be directly related to their tumor latent period in irradiated syngeneic hosts, regardless of species.

   b) The differential susceptibility occurred for neoplastic cell lines which did not differ from the nonneoplastic cell line in surface morphology in scanning electron microscopy, or in growth rates and saturation density in vitro.

   c) Two human choriocarcinomas were no more susceptible than mouse or human nonneoplastic cell lines. This resistance appeared associated with the inability of the activated macrophage to come into intimate contact with the surface of the neoplastic cells.

   d) The increased translational motion of BCG-activated macrophages while killing neoplastic cells was shown to be neither necessary nor sufficient. Cortisone inhibited the killing without changing the motion, cytochalasin B inhibited the killing with a decrease in motion, and Con A stimulated the killing in the absence of increased motion.

   e) The susceptibility to killing by activated macrophages can be dissociated from lack of density inhibition of growth in a series of hybrids between L cell and HTC.

3. In seven different instances, neoplastic and nonneoplastic cell lines were both derived from a common origin (single cell, minced embyro pool, or continuous cell line).

a) Nonneoplastic cell lines showed extensive cytoplasmic microtubules and thick actin cables. Neoplastic showed few actin cables, and microtubules were visible only in relatively well spread neoplastic cell lines.

b) Polarization microscopy confirmed these patterns in living cells. Nonneoplastic cells had birefringent actin cables and neoplastic cells had birefringent microtubules in processes.

c) Spreading of trypsinized nonneoplastic cells on glass revealed three stages:
   1) spreading of structureless cytoplasm,
   2) later growth of cytoplasmic microtubules,
   3) appearance of thick actin fibers if cell becomes elongated, coincident with locomotion of the cell.

   These structural changes may be causally related to or a passive consequence of changes in cell geometry while spreading on glass.

4. Nonneoplastic cell lines did not produce colonies in agarose while neoplastic cells produced colonies whose numbers and size were not related to tumor latent period. The plating efficiency of spontaneously transformed cells in agarose was lower than that of virally transformed cell lines described in the literature.

Significance and Proposed Course:

1. During spontaneous neoplastic change, saturation density and growth rate in vitro may change after the neoplastic change.

2. Human choriocarcinoma is the first neoplastic cell line resistant to killing by BCG-activated macrophages. This resistance to killing may be related to the trophoblast origin of the cell line. The lack of contacts between the macrophage and this resistant cell line suggests that intimate contact with the target cell is an absolute requirement for killing by activated macrophages. However, contact is only one step in the killing process, since cortisone inhibits killing without changing contact viewed in the light microscope.

3. If the structural changes (actin cables and microtubules) are merely passive consequences of changes in cell geometry, then other functional entities (e.g., adhesive sites on membrane) must be the cause for the different morphology of the neoplastic cell. If the actin fibers and microtubules are the cause of the morphological changes, the control of these structures by the cell may also be related to other functional aspects of the neoplastic cell, such as growth in agarose.

4. The fact that spontaneously transformed cell lines appear to grow in agarose less well than virally transformed cell lines suggests that either

spontaneously transformed cell lines have inherently lower plating efficiency on any surface, or they have changed from the nonneoplastic cell less than the virally transformed cells. Certainly, a very low plating efficiency in agarose is consistent with growth as a progressive tumor in vivo.

Publications:

1. Meltzer, M., Tucker, R.W., and Breuer, A.C.: Interaction of BCG-activated macrophages with neoplastic and nonneoplastic cell lines in vitro: Cinemicrographic analysis. Cell. Immunol. 17: 30-42, 1975.

2. Meltzer, M., Tucker, R.W., Sanford, K.K., and Leonard, E.J.: Interaction of BCG activated macrophages with neoplastic and nonneoplastic cell lines in vitro: Quantitation of the cytotoxic reaction by release of ^{3}H-thymidine from prelabeled target cells. J. Natl. Cancer Inst. 1177-1184, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5228-02 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Cytology of Neoplastic Transformation In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. L. Handleman	Biologist	LB NCI
OTHER:	K. K. Sanford	Research Biologist	LB NCI
	G. M. Jones	Microbiologist	LB NCI
	C. U. Garrison	Technician	LB NCI

COOPERATING UNITS (if any)

Dr. Lewis Lipkin, DCBD, NCI
Dr. Robert Tarone, Biometry Branch, NCI

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to identify and quantify cytologic characteristics of cells in culture that are associated with their neoplastic transformation by chemical carcinogens, as well as for following the progressive, often very subtle changes associated with the spontaneous neoplastic transformation of cells in vitro.

PHS-6040
(12-75)

Methods Employed: A combination of cytologic criteria previously described and validated for C_3H mouse cell cultures is used to study individual cells at high power as well as colony morphology at low power. Lambda-sized drops of cell suspension are used to quantify cell migration and orientation. Each lambda-drop contains 1000 cells. The drops are placed in T25 flasks for 1-3 hours to allow attachment of cells to the flask floor. After this settling period, culture medium is added to the flasks. Flasks are then incubated for 24 hours, fixed, stained, and drops photographed for analysis.

Cytologic Studies on "Spontaneously" Transformed Cells:

Study 1: The cytology of cells undergoing neoplastic transformation in culture. The objective is to study the progressive cytologic changes of embryo cells of human, rat, hamster and mouse origin during long-term culture and to establish more quantitative, objective cytologic criteria for neoplastic transformation of cells in vitro and a systematic approach to in vitro diagnosis of neoplastic state. This study was outlined in last year's annual report.

In an attempt to make some definite conclusions about the applicability and feasibility of quantifying certain morphologic properties of colonies in vitro, 24 hour lambda-drop preparations are being anlyzed. These 24 hour lambda-drop colony preparations are the product of spreading and migration of cells from a drop of cells derived from a trypsinized monolayer culture. It appears that there is a correlation between the progressive loss of parallel orientation of cells out of a lambda-drop and increasing tumorigenic potential. Also, it appears that cells of increased tumorigenic potential migrate farther than cells of low tumorigenic potential during equivalent periods of time.

By photographing each lambda-drop colony, and using a computer graph-pen to mark the long axis of each cell, it is possible with the use of a computer to measure the angle of each cell relative to the radial axis of the colony as well as determining the distance of each cell from the origin of the colony. By this means it is hoped that cell orientation and migration can be measured accurately from quantitated cell samples and used to diagnose neoplastic state.

The photographs of the lambda-drop colonies can be used to measure cell migration in another way. The method is as follows: photograph the lambda-drop colonies of a pair of nonneoplastic and neoplastic cell lines; determine which is the largest colony; draw the circumference of that colony; draw 3 additional circles equi-distant from the colony origin along the radius; draw these 4 circles on all the photos from the pair of cell lines; count the number of cells in the outermost zone on all the photos. By this method, it is possible to detect differences in migration rates of nonneoplastic and neoplastic ell line pairs.

More data and statistical interpretation of the data are required before the validity of these methods of quantifying cell populations can be established.

Study 2: The cytology of rat liver epithelial cells.

The objective of this study is to identify cytologic characteristics of cultured rat liver epithelial cells that may be directly or indirectly associated with their neoplastic state. Because the cytologic criteria of malignancy as applied to fibroblast-like cells in culture may not be applicable to cultured epithelial cells, several sets of nonneoplastic and carcinogen transformed rat liver epithelial cells have been prepared for cytologic study using 24 hour scraped preparations and 24 hour lambda-drop preparations. To date 16 cell lines have been studied. Diagnoses were consistant with the in vivo assays in 15 of the lines, with one questionable diagnosis. It seems, therefore, that many of the cytologic and morphologic criteria previously described and validated for C_3H mouse fibroblasts are applicable to rat liver epithelial cell cultures. Several more rat epithelial cell lines will be studied to further confirm this observation.

Other Activities of the Past Year Were:

1. The development of a line of rat liver epithelial cells.

The objectives of this study were: To develop a method for setting up cultures of epithelial cells from adult rat livers and determine the culture conditions needed for their optimum growth; to collect and save the supernatant medium from the cultures and see how its composition changes during long-term culture; to apply chemical carcinogens to the cultures and follow the metabolism by the cells.

This study was outlined in last year's report, cells were cryopreserved and the study has been terminated. The results of this study indicated that this rat liver cell line metabolized carcinogens in a manner characteristic of liver paranchymal cells and liver microsomal preparations. Further studies with these cells are temporarily suspended.

2. The development of a rapid routine chemical assay to test cell cultures for the presence of PPLO.

This project was outlined in last year's annual report. The validity of the test has not been confirmed, and the test is, therefore, not used routinely.

Proposed Course: Studies 1 and 2 will be completed for publication.

Significance: One of the problems in using morphologic and cytologic criteria of neoplastic transformation is the subjective nature of the evaluations, the lack of quantitation, and the difficulty in conveying to others the basis for judgement. We, therefore, in these studies are attempting to define quantifiable parameters to provide more objective means of in vitro monitoring, needed for our experimental studies on carcinogensis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5239-02 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Carcinomatous change in human cells studied in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Fox	Scientist	LB NCI
OTHER:	K. K. Sanford	Research Biologist	LB NCI
	F. M. Price	Research Biologist	LB NCI
	G. M. Jones	Microbiologist	LB NCI
	C. M. Garrison	Laboratory Technician	LB NCI

Cooperating Units: J. A. Dvorak, Lab. of Parasitic Diseases, NIAID: J. K. Selkirk, Chemistry Branch, NCI; D. H. Reese, Lung Cancer Branch, NCI; M. H. Cottler-Fox, Viral Oncology Branch, NCI; R. Parshad, Howard University Medical School; T. Caspersson, Karolinska Institutionen, Stockholm Sweden; L. Koss, Department of Pathology, Albert Einstein School of Medicine, New York; J. Jacobs, U. of Massachusetts Medical School, Worcester, Mass.

COOPERATING UNITS (if any)

See Above.

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Cell Physiology & Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Project Description:

Objective: To study the changes that occur in differentiated human epithelial cells accompanying transformation using morphological, chemical and physiological parameters. Further, to establish a system for transformation of human cells at will and to use such a system for estimating the likelihood of neoplastic transformation in vivo.

PHS-6040
(12-75)

Methods Employed: The methods to be employed will be described under each study heading.

Study 1. Neoplastic transformation of human epithelium in vitro.

The first phase of this study was an attempt to determine the effects of polycyclic hydrocarbon carcinogens on human epithelial cells in vitro, to investigate the differences in metabolism of benzo(a)pyrene (BP) in epithelial cells from different patients, and to contrast that metabolism with BP metabolism by cultured fibroblasts from the same donor.

Epidermal cells are being isolated into culture by Mr. F. M. Price using techniques he has developed over the past two years. We are now able to obtain growing epidermal cell cultures from human skin with remarkable regularity. These cultures are maintained as primary cultures which are entirely adequate for the types of studies we are now conducting. The skin cells are treated with a variety of carcinogens. At present we are using polycyclic hydrocarbons with our interest centered on benzo(a)pyrene, a carcinogen known to be widespread in the environment. A persistent problem has been in obtaining healthy human skin. Most of our supply has been furnished by local surgeons from patients undergoing plastic and reconstructive surgery.

It should be noted that human skin epithelium is one of the most difficult systems to study, both because it is extremely fastidious in its growth requirements, and because it is far more difficult to transform than other mammalian cells. This research is time consuming, tedious, and will be of long duration, but in the light of 85% of human cancer being due to carcinoma we believe that the difficulties are bearable and understandable.

Recent experiments in cooperation with Dr. J. K. Selkirk demonstrated that primary cell cultures derived from human skin epithelium metabolize benzo(a) pyrene to three classes of compounds: phenols, quinones and dihydrodiols. The relative proportions of metabolites varied according to the skin donor but differed from the pattern of metabolites in rat liver microsome preparations. While appreciable amounts of 7,8- and 9,10 dihydrodiol; 1,6-, 3, 6-, and 6, 12-quinone; and 3-and 9-hydroxy derivatives were found in the medium, no 4.5 (K-region) dihydrodiol or epoxide was detected. The cultures did not require a period of enzyme induction for efficient metabolism of the hydrocarbon and the carcinogen produced extensive injury and cell death. In contrast, cultures of fibroblasts derived from the same skin samples as the epithelial cells metabolized the hydrocarbon but to a different extent and showed little or no cytotoxic response. Pre-exposure of the epithelial cell cultures to mixtures of polycylic hydrocarbons resulted in a decrease in the amounts of carcinogen metabolized to phenols and dihydrodiols.

These findings suggest that the prevalence of carcinomatous disease in humans may be due to the differential capacity of epithelial cells to metabolize potential carcinogens to active forms, a capacity reduced in fibroblasts or

other nonepithelial cells. This suggestion is supported by the observation that human prostate cells also efficiently metabolized BP in a manner similar to the epidermal cells. No evidence of neoplastic transformation was seen in cytological preparations of cells exfoliated into the medium. If transformation is to be demonstrated in vitro, it seems likely that some means for reducing the toxicity must be found to avoid complete removal of the epithelium. These results demonstrate the variance of a BP metabolite profile in cells derived from humans and amplify the need to examine more closely carcinogen metabolism and activators in humans.

Studies on metabolism of BP and other polycyclic hydrocarbons on human epithelial cells will be continued with attention to cellular responses indicative of transformation.

Study 2. Morphology of transformation in human and rodent cells.

In collaboration with K. K. Sanford and J. A. Dvorak we have found that there are certain morphological characteristics of transformed rodent fibroblasts that can be quantitatively described using light microscopy. These characteristics include cell thickness, projected area, and relationship between cytoplasmic RNA and protein concentrations. In order to pursue and amplify these observations we have obtained the collaboration of Prof. T. Caspersson who has consented to allow us to use his integrating interferometer and automated microscopes, and with Dr. L. Koss who is providing us with pathologic interpretation of cells in conventional Papanicoulau preparations. Eight pairs of nonneoplastic and neoplastic cell lines, some of single cell origin, are currently under investigation in Prof. Casperssons laboratory. Differences in dry mass and other biochemical parameters will be evaluated and should provide important quantifiable criteria of transformation in cultured fibroblasts. Two additional parameters of neoplastic transformation in spontaneously transformed fibroblasts were examined: the predilection for rounding of cells at metaphase and the projected area of adherent cells 24 hours after plating on a cover glass. Ten different cell lines including those of hamster, rat and mouse origin, and cultures of known neoplastic potential as determined by tumor production in vivo were examined by time lapse cinephotomicrography for metaphase rounding. A close correlation between rounding of cells at metaphase and tumorigenic potential was observed. As the cell population became neoplastic, there was also a significant decrease in the mean projected area of the cells.

The decrease in projected cell area and rounding of rodent fibroblasts at metaphase may be related phenomena and reflect a fundamental difference between cell populations which produce tumors on injection into suitable animals and those that do not.

An independent study by Cherny et al (1975) reported a similar decrease in projected cell area with development of oncogenic potential, interpreted as due to a decrease in "lamellar cytoplasm". Possible interpretations including

amounts of plasma membrane elaborated, endoskeletal pattern, and adhesions to substrate may account for this basic morphologic difference between non-neoplastic and neoplastic cells and will be explored.

Miscellaneous Studies:

Three additional studies outlined in last year's report, effect of physical carcinogens, transformation of transitional epithelium, and regulation of lactic acid metabolism in cultured neoplastic and embryo cells have been continued. In addition, attempts to culture chicken liver cells have been pursued (see report by F. M. Price, 3212-02).

Publications:

1. Fox, C.H., Selkirk, J.K., Price, F.M., Croy, R.G., Sanford, K.K., and Cottler-Fox, M.: Metabolism of benzo(a)pyrene by human epithelial cells in vitro. Cancer Res. 35: 3551-3557, 1975.

2. Fox, C.H., Dvorak, J.A., Sanford, K.K.: Cytometric analyses of neoplastic transformation of vertebrate cell populations. Cancer Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5222-07 LB

PERIOD COVERED July 1, 1975 to June 20, 1976

TITLE OF PROJECT (80 characters or less)

Mutations in the first structural gene of the histidine operon which alter regulation of the operon.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. B. Meyers	Research Chemist	LB NCI
OTHER:	R. F. Goldberger	Medical Officer (Chief)	LB NCI
	M. Levinthal	Dept. of Biol. Sciences	Purdue University

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Cellular Regulation Section

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated

PHS-6040 (12-75)

Project Description:

Objectives: On the basis of previous work done on the kinetics of repression of the histidine operon in Salmonella typhimurium, we suggested that the first enzyme for histidine biosynthesis plays a previously unrecognized role in control of the histidine operon. A prediction of this hypothesis is that certain mutations in the first structural gene (G gene) of the operon would lead to alterations in the structure of the first enzyme which affect regulation of the histidine operon. Although a number of mutations affecting the catalytic function of this enzyme are known, none of these affects the process of repression. We have been attempting to find mutations in the first structural gene which do alter the repression process.

Methods Employed: We developed a procedure which allowed the direct selection of feedback resistant mutations in the G gene of episomes carrying the histidine operon. Twenty feedback resistant episomes were isolated. Several were found to produce a G enzyme highly resistant to inhibition by histidine. Using these episomes to make merodiploid organisms, we were able to demonstrate that the effect of the G enzyme on repression of the histidine operon was trans dominant (see Project Report NIAMDD-LCB-14). The feedback resistant episomes is not under repression control. This is the first isolation of the consistitutive G gene mutations predicted by our hypothesis that the G enzyme plays a direct role in repression of the histidine operon.

This episome has been integrated into the chromosome of several strains carrying deletions of the histidine operon. The resulting strains exhibit the same patterns, i.e., the levels of the histidine enzymes are approximately 4X derepressed and are not under histidine control. The episome carrying the altered G gene has been transduced into his T and his W mutants of Salmonella and in all cases the enzyme levels are fixed at the 3X level. The same result was obtained when the episome was transduced into an E. coli strain which had the his operon deleted.

Major Findings: An episome bearing the histidine operon containing a mutation to feedback resistance has been isolated. The strain carrying this episome is not under repression control. This finding supports the hypothesis that the G enzyme plays a direct role in repression of the histidine operon.

Significance to Cancer Research: Alterations in the first enzyme for histidine biosynthesis which affect repression of the histidine operon constitute proof of our hypothesis that a biosynthetic enzyme may have regulatory properties which allow it to control its own synthesis as well as the synthesis of the other biosynthetic enzymes encoded in the same operon.

Proposed Course of Research: Terminated (October 1975).

Publications:

Meyers, M., Levinthal, M., and Goldberger, R.F.: trans-Recessive mutation

in the first structural gene of the histidine operon that results in constitutive expression of the operon. J. Bacteriol. 124: 1227-1235, Dec. 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CBD 5225-07 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Interaction between the first enzyme for histidine biosynthesis and Ø80dhis DNA.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. B. Meyers	Research Chemist	LB NCI
OTHER:	T. Vogel	Visiting Associate	LB NCI
	R. G. Deeley	Visiting Associate	LB NCI
	R. F. Goldberger	Medical Officer (Chief)	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cellular Regulation Section

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated

Project Description:

Objectives: We previously proposed that the first enzyme for histidine biosynthesis, the G enzyme, plays a role in regulation of the histidine operon. If this enzyme acts at the genetic level, it would be expected to interact with some regulatory element in the DNA of the histidine operon. The recent isolation of a phage carrying the histidine operon in place of some of its own genes has made it possible to test this possibility.

Methods Employed: Radioactively labeled and unlabeled DNA were isolated from Ø80dhis. Interactions between these preparations of DNA and the G enzyme were studied by the filter binding assay used by Riggs and Bourgeois to study the binding of the lac repressor to the lactose operon. Competition experiments were done using cold Ø80DNA, cold Ø80dhis DNA, and cold Ø80dhis carrying a deletion mutation in the operator region of the his operon, to determine if these preparations of DNA also bind to the G enzyme.

Major Findings: The enzyme binds Ø80dhis DNA. This binding is completely inhibited by cold Ø80dhis DNA. However, cold Ø80DNA does not inhibit, and the cold Ø80dhis operator deletion DNA does not inhibit. These results indicate that the G enzyme binds to the regulatory region of the his operon DNA.

Significance to Cancer Research: The finding that an enzyme may serve as a regulatory protein, controlling the rate of its own biosynthesis, may be of general importance in understanding the mechanism of repression control.

Proposed Course of Research: Terminated (July 1975).

Publications:

Meyers, M., Blasi, F., Bruni, C.B., Deeley, R.G., Kovach, J.S. Levinthal, M., Mullinix, K.P., Vogel, T., and Goldberger, R.F.: Specific binding of the first enzyme for histidine biosynthesis to the DNA of the histidine operon. Nuc. Acids Res. 2: 2021-2036, Nov. 1975.

Goldberger, R.F., and Deeley, R.G.: Autogenous regulation of gene expression. In Marzluf, G.A. and Copeland, J.C. (Eds.) Regulatory Biology, Ohio State University Press, Columbus (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5238-07 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Estrogen on Gene Expression in the Avian Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. F. Goldberger	Medical Officer (Chief)	LB NCI
OTHER:	K. P. Mullinix	Research Chemist	LB NCI
	R. G. Deeley	Visiting Associate	LB NCI
	M. B. Meyers	Research Chemist	LB NCI
	W. Wetekam	Visiting Fellow	LB NCI
	J. Gordon	Research Associate	LB NCI
	K. Kent	Chemist	LB NCI
	J. D. Eldridge	Biologist	LB NCI
	M. R. Dhawale	Visiting Fellow	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Cellular Regulation Section

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	4.5	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

The work described herein consists of experiments designed to educate the mechanisms by which estrogen induces the synthesis of vitellogenin, the precursor of the egg yolk phosphoproteins, in rooster liver. Vitellogenin mRNA has been partially purified from rooster liver and has been shown to have a size of approximately $2x10^6$ daltons. Vitellogenin mRNA is not present in normal roosters whereas it is synthesized and accumulates in the liver in response to estrogen stimulation. In addition vitellogenin mRNA can be isolated from roosters no longer actively synthesizing vitellogenin after estrogen withdrawal in amounts equal to appriximately 30% of that found in the fully induced state. Vitellogenin mRNA will be purified to homogeneity and used as a template to synthesize complementary DNA. This DNA will be used to study the synthesis of vitellogenin mRNA in vitro and in vivo.

PHS-6040
(12-75)

Project Description:

Objectives: To elucidate the mechanisms by which estrogen regulates the synthesis of vitellogenin, the precursor of the egg yolk phosphoproteins, lipovitellin and phosvitin, and to determine the mechanism by which vitellogenin is cleaved to yield lipovitellin and phosvitin.

Methods employed: Vitellogenin mRNA, synthesized in rooster liver in response to estrogen, has been extracted and identified by its ability to direct the synthesis of vitellogenin in vitro in the rabbit reticulocyte cell-free protein synthesis system. Specific immunoprecipitation techniques, employing highly purified monospecific antibodies, have been used to identify vitellogenin as a product of the cell-free synthesis reaction. Sucrose gradient centrifugation has been used to analyze polysomes extracted from the avian liver, and analysis of RNA extracted from various size classes of polysomes has been conducted in order to determine the characteristics of vitellogenin-synthesizing polysomes at different times after estrogen stimulation. A variety of techniques have been developed to extract large quantities of intact vitellogenin mRNA. This vitellogenin mRNA has been partially purified by centrifugation and affinity chromatography. Translation of small amounts of vitellogenin has been tested by the techniques of microinjection of frog oocytes.

Major Findings: Vitellogenin mRNA has been isolated and characterized. It has been demonstrated that this mRNA has a size of approximately $2x10^6$ daltons, which is the size expected of a mRNA that directs the synthesis of vitellogenin, a protein having a polypeptide molecular weight of 220-240,000. Vitellogenin mRNA, assayed by its ability to direct the synthesis of vitellogenin in vitro, is not present in liver of normal roosters but its synthesis is induced in rooster liver by treatment with estrogen. After an initial exposure to estrogen, the synthesis of vitellogenin mRNA is accompanied by the synthesis of the protein, vitellogenin, and the synthesis of this protein persist for several days after exposure to estrogen, after which vitellogenin synthesis ceases. It has been demonstrated that even after vitellogenin synthesis ceases in roosters after a primary, secondary or tertiary exposure to estrogen vitellogenin mRNA can be isolated from rooster liver in amounts corresponding to approximately 30% of that found in liver of estrogen-stimulated roosters that are actively synthesizing vitellogenin. It has been demonstrated that whereas vitellogenin mRNA in estrogen stimulated roosters that are actively synthesizing vitellogenin is associated with the largest sizes of polysomes extracted from the liver, vitellogenin mRNA in roosters that have been estrogen stimulated but are no longer synthesizing vitellogenin is not associated with polysomes. Vitellogenin mRNA in the latter roosters is associated with either mRNP particles, ribosomal subunits or with monosomes.

Significance to Cancer Research: It can be assumed that at least some forms of cancer can be attributed to aberrations in the mechanisms that control gene expression. At present we have minimal knowledge of the processes that control the expression of genes at the transcriptional or the translational level in either the normal or cancerous cell. One possible way to study these processes is to examine biochemically the ways in which certain agents can effect specific gene expression. An ideal system for these studies is one in which hormones that can regulate the synthesis of specific proteins are employed. In the studies described above estrogen is used to induce the synthesis of vitellogenin in the rooster, an animal in which the vitellogenin gene is completely repressed in the normal condition. In addition, the synthesis of vitellogenin is appropriate for the study of controls of regulation of post-translational modification of proteins since vitellogenin is a protein that is phosphorylated and glycosylated after or during its translation and it is presently not known how or to what extent post-translational modifications influence specific protein synthesis and transport.

Proposed Course of Research: Vitellogenin mRNA will be purified to homogeneity and techniques will be developed to utilize reverse transcriptase to copy this mRNA to yield complementary DNA. This "probe" DNA will be used to quantitate amounts of vitellogenin mRNA present in rooster liver at various times after estrogen stimulation. This "probe" DNA will also be used to monitor vitellogenin mRNA synthesis *in vitro* using chromatin under a variety of conditions. The phenomenon of the existence of non-translated vitellogenin mRNA in estrogen-withdrawn roosters will be further studied in order to determine whether this mRNA is material that is long-lived and remains in the cell after estrogen stimulation and withdrawal or is constantly synthesized at a low level after estrogen withdrawal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 5240-02 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The synthesis of vitellogenin by avian liver cells in culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. G. Deeley	Visiting Associate	LB NCI
OTHER:	J. D. Eldridge	Biologist	LB NCI
	R. F. Goldberger	Medical Officer (Chief)	LB NCI
	C. H. Fox	Senior Scientist	LB NCI
	F. Price	Research Biologist	LB NCI
	J. H. Highland	Staff Fellow	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Cellular Regulation Section

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves the establishment of an avian liver cell culture system and its use in the study of the mechanism of induction of the egg yolk phosphoprotein, vitellogenin, by estradiol.

Project Description:

Objectives: To establish a primary avian liver cell culture system in order to further elucidate the mechanism of estradiol induction of the egg yolk phosphoprotein, vitellogenin.

Methods Employed: Organ cultures have been prepared from the finely minced livers of nine day old chicks and have been maintained in culture for several weeks in a synthetic medium supplemented with 10% fetal bovine serum. In these initial experiments the use of proteolytic enzymes, to facilitate the dispersal of individual cells, has been avoided because of the possibility of destroying estrogen receptors on the cell surface.

Experiments have been carried out with cultures made from the livers of chicks which have been treated with estrogen and which were synthesizing vitellogenin when sacrificed. Similar cultures have also been prepared from the livers of untreated chicks. The cultures made from the livers of estrogen treated chicks have been maintained in a medium containing 10^{-6} M estradiol, while the cultures from untreated chicks have been maintained in a similar medium both in the presence and absence of estradiol. The polypeptides synthesized by these cultures at various times during the first two weeks in culture have been labelled with ^{3}H-leucine and $^{33}PO_4$. The radioactively labelled polypeptides secreted into the medium have been examined by electrophoresis on acrylamide gels containing SDS and by various immunological techniques.

Major Findings: Chicken liver cultures prepared in the fashion outlined above continue to synthesize and secrete polypeptides for several weeks; during this period some cell division occurs. The major secreted polypeptide has the same electrophoretic mobility on SDS-acrylamide gels as albumin. Cultures prepared from the livers of chicks which have been treated with estrogen continue to synthesize intact, phosphorylated vitellogenin for the first 24 to 48 hours in cultures. After approximately three days in culture, the synthesis of intact vitellogenin polypeptides (molecular weight 240,000) is not detectable. However, two polypeptides (molecular weights 180,000 and 30,000) which may be generated by proteolytic cleavage of intact vitellogenin continue to be synthesized. These polypeptides are being characterized immunologically. The synthesis of two similar polypeptides can be induced in cultures prepared from the livers of untreated chicks by the addition of 10^{-6} M estradiol to the culture medium. So far it has not been possible to detect the synthesis of intact vitellogenin in cultures prepared from the livers of untreated chicks.

Significance to Cancer Research: The mechanism by which the expression of particular genes is regulated remains, in large part, unclear. We hope that study of the molecular mechanism of gene expression in this system will provide a better understanding of the role of steroid hormones in such regulation.

Proposed Course of Research: Further experiments will be carried out to determine culture conditions necessary to induce and maintain the synthesis of intact vitellogenin in a primary cell culture. Experiments will then be designed to determine the half-life of vitellogenin mRNA and to examine the early kinetics of induction of vitellogenin synthesis, following primary and secondary exposures to estrogen. Complementary DNA prepared from purified vitellogenin mRNA will be used as a probe in these studies.

The cultured cells will provide a homogeneous source of chromatin and RNA polymerase which will facillitate in vitro studies on the molecular mechanisms involved in the regulation of vitellogenin synthesis. This system also offers the opportunity of comparing in a single cell type, the regulatory mechanisms of a hormonally inducible system, which is easily modulated, with those mechanisms involved in maintaining the expression of a terminally differentiated function such as the synthesis of albumin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5231-03 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Role of Subunit Interactions in Enzyme Chemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Klee	Research Chemist	LB NCI
OTHER:	J. Craine	Biochemist	LB NCI
	E. Barbehenn	Chemist	LB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	3.0	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

Project Description:

Objectives: To study the functional role of subunits and protein-protein interactions in two enzymatic systems: polynucleotide phosphorylase and cAMP phosphodiesterase.

Methods Employed: The project involves the study of properties of proteins, purified by the usual techniques of protein isolation, including many types of chromatography. The enzymes are being studied by examining their optical, hydrodynamic, and kinetic properties as well as by suitable chemical measurements and modifications.

PHS-6040
(12-75)

1) Polynucleotide phosphorylase

Structure-function relationships

The complex multi-banded pattern produced by PNase upon disc gel electrophoresis has now been partially clarified. There are two major sets of bands which have been shown to represent the 220,000 MW monomer and the 440,000 MW dimer as determined by Ferguson plots. Within either of these major sets, a complex subset pattern is seen. These subset bands have been characterized both as to their relative amounts as to RF values as measured by gel scans. Since enzyme run in both SDS and acid urea shows only one major species, the native subset patterns may be due to conformational isomers or to several types of enzyme-ligand complexes. This is further supported by our ability to modify the amounts of particular species by purification, storage (freeze/thaw), ionic strength and oligonucleotide addition. This latter effect is quite dramatic and varies as a function of chain length. Although no effect is seen when n = 1 or 2 in the series $(Ap)_n$ all higher members produce a characteristic electrophoretic mobility. These responses can be grouped as follows: n = 3 to 6, n = 25 - 90 and n 200. These groups of electrophoretic species may reflect different enzyme - oligonucleotide complexes.
(E. Barbehenn & C. Klee).

Mechanism of action

The mechanism of initiation of polynucleotide synthesis is being studied using deoxy-ADP as a substrate. During the course of these studies it was found that adenylate kinase activity is closely associated with polynucleotide phosphorylase and cannot be removed by fractionation based on size or charge. The possible role of this enzymatic activity in the mechanism of initiation of polynucleotide synthesis is being investigated. (J. Craine, C. Klee)

2) Regulation of cAMP phosphodiesterase

The cAMP phosphodiesterase activator protein has been purified to homogeneity in a new procedure with high yields. The protein has been characterized by its amino acid composition, its electrophoretic properties under native and denaturing conditions, and its spectrophotometric characteristics. The purified activator has been used to design a purification procedure for the soluble cAMP phosphodieterase based on affinity chromotography. The enzyme obtained by this technique is seen to be at least 80% pure by disc gel electrophoresis.

Ca^{2+} binding is the first step in the regulation of cAMP phosphodiesterase by its activator. Ca^{2+} binding to the activator has been studied by equilibrium dialysis. The activator protein contains 4 nonequivalent or interacting sites for Ca^{2+} with association constants varying between 0.1 and 0.6 x 10^6. Binding of Ca^{2+} induces a conformational change resulting in a 20% increase in molar elliplicity at 222 nm and a 10% decrease in the

tyrosine and phenylalanine absorption bands. All these changes are reversible with EGTA. Interaction of the activator-Ca^{2+} complex with the enzyme is greatly affected by cAMP concentration. High cAMP levels result in a 10 fold decrease in the K_m for activator ($0.5 - 5 \times 10^{-9}$ M activator). These effects of cAMP on activator stimulated activity are similar to those observed on imidazole stimulated activity which is presently being studied. (C. Klee)

Significance to biomedical research and the program of the Institute:

Both proteins being studied are examples of regulation of catalytic activity by protein-protein interactions. Polynucleotide phosphorylase, a well-known bacterial enzyme, also has been described in mammalian cells where its phosphorolytic activity alone has been observed. It is widely utilized for the synthesis of model nucleic acids used in nucleic acid enzymology and interferon induction, and also in the degradation of poly A sequence of mammalian mRNA. Its physiological role is still undertermined, but it would seem likely that the enzyme has an important function in nucleic acid metabolism.

cAMP phosphodiesterase, as one of the enzymes responsible for the control of cAMP levels which are critical for the regulation of cell growth and differentiation, is of considerable interest. Its regulation by endogenous proteins and other factors is undoubtedly of great importance to the cell. The molecular features of this regulation need to be elucidated.

Proposed course of research;

Studies on polynucleotide phosphorylase will be directed towards the elucidation of the nature of subunit interactions leading to the multiple enzymatic species. The loss of primer independence upon trypsin treatment will be used as a tool to study the mechanism of initiation of polynucleotide synthesis catalyzed by the enzyme.

Control of phosphodiesterase activity by the naturally occurring protein activator will be studied further and compared with the positive and negative control of activity effected by other types of activators such as imidazole, or inhibitors such as theophylline. The kinetics of this enzyme system seem very complex and efforts will be made to simplify this picture by working with monomolecular enzyme species. The potential role of subunit interactions in the apparently negative cooperative kinetics observed will also be studied. Subcellular localization of the components of this system will be studied with the aid of antibodies currently being prepared.

Publications:

1. Klee, C.B., Kirk, K. L., Cohen, L. A., and McPhie, P.: Histidine ammonia-lyase: the use of 4-fluorohistidine identification of the rate-determining step. J. Biol. Chem. 250: 5033-5040, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 5232-03 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Replication of Simian Virus 40 (SV40) DNA in Monkey Kidney Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Kay	Visiting Fellow	LB NCI
OTHER:	M. F. Singer	Research Chemist	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Development of an in vitro replication system in order to study the enzymatic mechanism of SV40 DNA replication. Study of the relation between histone synthesis and viral DNA synthesis.

PHS-6040
(12-75)

Major Findings: There has been no significant progress made this past year in the further development of the *in vitro* system for studying SV40 DNA synthesis. Work has centered on obtaining a system that completes replication of SV40 DNA in a reproducible manner.

Preliminary findings indicate that the synthesis of all 5 major histone fractions is stimulated upon initiation of SV40 DNA synthesis in infected cells.

Significance to Cancer Research: Efforts on the *in vitro* system for synthesis of SV40 DNA have been discouraging. While some continuing effort to obtain a reproducible system is anticipated, our main effort in the coming year will be related to synthesis of histones.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5234-03 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Replication and Properties of Defective-Substituted Simian Virus 40 in Permissive Monkey Kidney Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. R. K. Rao	Visiting Fellow	LB NCI
OTHER:	F. Blasi	Visiting Scientist	LB NCI
	S. Segal	Visiting Fellow	LB NCI
	A. Kay	Visiting Fellow	LB NCI
	M. Fox	Microbiologist	LB NCI
	M. F. Singer	Research Chemist	LB NCI

COOPERATING UNITS (if any)

1. Martin Rosenberg, Ph.D., Lab. MBGY DCDB/NCI
2. Margaret Johnson, Section on Biochemistry of Gene Expression, LB

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.9	0.8

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of the mechanism whereby defective viral DNA is synthesized preferentially to non-defective DNA. Studies on the structure of defective SV40 genomes, with emphasis on defectives that are substituted with host (monkey) sequences. Origin and nature of the host sequences incorporated into defective SV40.

PHS-6040
(12-75)

Objectives: Serial, undiluted passage of Simian Virus 40 (SV40) yields defective virions containing DNA which is 1) shorter than normal, 2) partially substituted with host sequences. We wish to study the preferential replication and structure of such defective-substituted DNA as well as the nature of the host DNA sequences incorporated into the SV40 genome

Methods Employed: Standard tissue culture procedures are used for the growth of monkey kidney cells and SV40. Procedures used for studying viral DNA synthesis included radioisotope tracer techniques, preparative and analytical ultracentrifugation, DNA-DNA hybridization, selective precipitation of high molecular weight DNA, polyacrylamide and agarose gel electrophoresis, degradation with specific restriction endonucleases. Radioisotope tracer methods are essential. Electron microscope is used to study the size and configuration of DNA and is also used to study DNA structure by heteroduplex analysis.

Major Findings: The rate of synthesis of viral DNA after infection of permissive monkey kidney cells with wild type and defective simian virus 40 was studied. The defective virus used is characterized by extensive substitutions of viral sequences with host DNA. Conditions were defined under which the rate of viral DNA synthesis is constant and dependent on the multiplicity of infection. Stocks of defective virus induce rates of DNA synthesis that reflect the concentration of plaque forming units in the stock. Infection with highly defective virus results in the continuing production of viral DNA and plaque forming units at a very slow rate over a period of many days, but virion particles were not detectable by the procedures used.

Mixed infection by plaque-purified viral stocks and defective stocks results in inhibition of the rate of DNA synthesis found after infection with the plaque-purified virus alone. The inhibition shows a marked specificity. Thus, it is greatest when the defective is derived from strain 777 and wild-type plaque purified 777 is used while inhibition by the same defective is far less when the wild type virus is strain 776.

The latter finding stimulated us to initiate the investigation of possible differences between the two widely used "wild type" SV40 strains (777 and 776). Digestion of 777 and 776 DNA with restriction endonuclease Hind(II+III) revealed that the genomes differ in the size of one particular known fragment, the "F" fragment. The 777 "F" fragment is about 25 base pairs shorter than the 776 "F" fragment. The location of the difference between the two genomes was confirmed by electron microscopic studies on heteroduplexes between linear forms of the two DNAs. The full length linear molecules were generated by cleavage with restriction endonuclease Hap II. Other workers have shown that the sequences of the "F" fragment code for a portion of the capsid protein of SV40. Therefore, studies on the structure of the two capsid proteins have been initiated.

Last year's report described the origin of a particular defective SV40 genome. Briefly, it derived from high multiplicity serial passages of 777 virus, and was characterized by a high degree of substitution by sequences derived from

host (monkey) repetitive DNA, and resistance to restriction endonuclease Eco R_I. We have now elucidated further the structure of this defective virion (now called CVP8/1/P2).

The structural analysis depends on hybridization studies and on study of DNA fragments generated by a variety of restriction endonucleases. Data presently in hand are consistent with the following type of structure. At least 25% of the total sequences are derived from host rather than wild type SV40 DNA. Of these, about 10% are from repetitive host DNA. The entire genome is composed of three identical DNA sequences (each about 23.5 percent of a full wild type genome) and one DNA sequence (about 25 percent of a full wild type genome) similar, but not identical to the other three. Thus, the host sequences, as well as remaining SV40 sequences are each repeated in 4 separate places around the circular DNA. The host sequences appear to be clustered together in each quadrant of the molecule.

A fragment containing all the host repetitive DNA of CVP8/1/P2 is generated by cleavage with restriction endonucleases Hind(II+III). These particular sequences are known to arise frequently in independently isolated SV40 defectives. Therefore, it appears likely that the sequences may represent a favored region for recombination of SV40 and host DNA and we have, for that reason, concentrated some effort on studying the fragment.

Careful analysis confirmed the presence of few, if any, SV40 sequences. The current estimates indicate that the fragment is about 140 base pairs in length. Primary oligonucleotide maps of RNA transcripts of the fragment have been complated and the fragment can thus be described as a particular family of sequences. Studies directed toward the ordering of the oligonucleotides are underway and we anticipate having the complete nucleotide structure in hand within the next year.

The location of the sequences in the fragment within the monkey chromosomes is being investigated by the in situ hybridization technique. The sequence is present on about half the monkey chromosomes (never on the X and Y chromosome). It is not limited to the centromeric region (as is true of typical satellite DNA in other species) but is found in non-centromeric regions.

Significance to Cancer Research: Simian Virus 40 is an oncogenic DNA virus known to produce tumors in hamsters. SV40 and related viruses have recently been identified in association with several human diseases, including a few tumors. Thus, any experimental evidence shedding light on the mode of replication of this virus is of obvious significance to NCI research. Further studies on substituted defective SV40 have 3 broad implications:

First, they raise the possibility of transduction experiments in animal systems, and second, the formation of the substituted molecules provides an interesting approach to the metabolism of DNA, in particular, to recombination. Third, defective animal virions occur upon passage of many viruses, and it has been that they play a part in the self-limitation of viral infections.

Proposed Course of Research:

1. The differences between strains 777 and 776 will be investigated further. In particular the structures of the two capsid proteins will be compared. Given the likely differences in the capsid proteins, and the interesting observation that defectives derived from 777 inhibit wild type 776 replication, it appears that a comparison between the two strains regarding absorption and penetration into permissive cells is of interest. Such studies are being planned.

2. Structural analysis of the Eco R_I endonuclease resistant, substituted defective genome will be completed. Analysis of the Eco R_I sensitive DNA present in CVP8/1/P2 will be initiated.

3. The work on the nucleotide sequence of the host repetitive DNA present in in the Eco R_I resistant defective will be continued. The frequency of the sequence within the monkey genome will be investigated.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5237-03 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Interaction of Histones With Superhelical DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. S. Singer	Staff Fellow	LB NCI
OTHER:	M. Bina-Stein	Staff Fellow	LB NCI
	M. C. Fox	Microbiologist	LB NCI
	M. F. Singer	Research Chemist	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Biochemistry

SECTION Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: 2.9	PROFESSIONAL: 2.4	OTHER: 0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of interaction between mammalian histones and superhelical DNA in vitro.

Objectives: To study the interaction between mammalian histones and superhelical DNA in vitro and relate the interaction to the functional role of histones in DNA structure and metabolism. Emphasis is placed on SV40 DNA as a model for superhelical DNA.

PHS-6040
(12-75)

Methods Employed:

Standard tissue culture techniques for growth of monkey kidney and mouse or human cells and for preparation of simian virus 40(SV40) DNA. Subcellular fractionation, protein purification, radioisotope tracer techniques, gel electrophoresis, ultracentrifugation, nitrocellulose filter binding techniques, protein chemistry and amino acid analysis; enzymic techniques (involving polynucleotide ligase or "unwinding enzyme"), coupled with agarose gel electrophoresis for isolation and analysis of closed-circular duplex DNAs of known superhelicity; electron microscopic visualization of superhelical DNA and bound histones.

Major Findings:

Earlier we found that the extent of interaction of H1 (formerly f1) histone with superhelical DNA increases with increasing superhelicity regardless of whether the superhelicity is of the positive or negative sense. These measurements depended on the presence of the intercalating dye, ethidium bromide, in the reaction mixture, to generate molecules of varying superhelicity. While the results appeared unambiguous, we were interested in confirming the observations with molecules of known and varying superhelicity, in the absence of ethidium.

We prepared a series of molecules of known superhelicity by one of two methods. In one, nicked circular duplex SV40 DNA was sealed by polynucleotide ligase in the presence of varying concentrations of ethidium bromide thus producing molecules of varying negative superhelicity upon removal of the ethidium. Similarly, incubation of SV40 DNA with "unwinding enzyme" in the presence of varying concentrations of ethidium produces molecules of varying negative superhelicity upon removal of the ethidium. In both instances the reactions produce molecules of zero superhelicity, that is, relaxed circular duplexes (DNA I^r) in the absence of ethidium.

Using these molecules we confirmed the earlier interpretation that the extent of interaction between H1 histone and closed circular duplex DNA depends on the degree of negative superhelicity. The more negatively superhelical the molecule, the less H1 is required to complex the DNA, as measured by the nitrocellulose filter binding technique.

We have also found that the special ability of H1 to complex superhelical DNA holds not only for H1 isolated from calf thymus, but also for H1 isolated from monkey and human cells.

Further, a special histone called H5 (previously called H2C) and found exclusively in mature avian erythrocytes where it replaces the bulk of the H1 histone, binds to superhelical DNA with the same specificity as H1 histone.

We have examined the H1 molecule itself and determined certain structural components that are required for specific binding with superhelical DNA. H1 has about 212 amino acid residues. Procedures published by others permit the

preparation of pure polypeptide fragments representing residues 1 through 71, 1 through 106, 73 through 212 and 107 through 212 respectively, where residue 1 is the NH_2 - terminus and residue 212 is the carboxy terminus. Amino terminal fragments 1 - 71 and 1 - 106, do not bind to superhelical DNA or, as shown by others, to linear DNA. The fragment 73-212 binds to closed circular duplex DNA with the same specificity as does H1 itself, namely, interaction with superhelical DNA is much more efficient than interaction with relaxed DNA 1[r]. The fragment 107-212 binds efficiently to DNA, but shows no preferential interaction with superhelical DNA. Thus, the region between residues 72 and 106, which is known to be within the only globular portion of the H1 molecule appears to be involved in the special reaction with superhelical DNA.

H1 molecules specifically phosphorylated at residue 37, or residue 105, or at multiple sites in the carboxy terminal half of the molecule were compared with unphosphorylated H1 for interaction with superhelical and relaxed closed circular duplexes. At our standard salt concentration (0.1 M) the preferential complexing of superhelical DNA was maintained in all cases. Preliminary results suggest that at lower salt concentrations interesting differences in the capacity of the phosphorylated H1 molecules to complex the DNA molecules may exist.

We have examined the influence of H1 histone on the ability of "unwinding enzyme" to unwind closed circular duplex DNA. Interaction of superhelical DNA and H1 histone protects the DNA from the action of unwinding enzyme. As the concentration of H1 is increased from zero, less and less relaxed DNA product is formed, and molecules of superhelicity in between zero and that of the initial substrate (about 22 negative superhelical turns) appear. Finally, at about 0.3 parts of H1 to 1 of DNA (by weight) none of the superhelical DNA is relaxed by "unwinding enzyme". Treatment of complexes of relaxed closed circular DNA and H1 with "unwinding enzyme" does not introduce superhelical turns into the DNA. Thus, interaction of duplex DNA with H1 does not involve unwinding of the duplex DNA structure.

Electron microscopic visualization of complexes between H1 histone and superhelical DNA has been initiated.

Significance to Cancer Research: The role of histones in 1) the structure of chromatin and 2) the regulation of gene expression and DNA replication in eukaryotic cells is under intensive investigation in many laboratories. The relevance of the control of eukaryote gene expression to the nature of oncogenic processes is evident.

Proposed Course of Research:

1. Investigate further the nature of the interaction and stoichiometry of the reaction between H1 histone and superhelical DNA. This will require procedures other than the nitrocellulose filter binding procedure. There are technical problems which will need intensive investigation before meaningful results can be obtained.

2. Investigate the nature of the apparent inhibition of "unwinding enzyme" by H1 histone. Is it caused by protection of the DNA, or by inhibition of the "unwinding enzyme"?

Publications

1. Chou, J. Y., Singer, M. F., and McPhie, P.: Kinetic studies on the phosphorolysis of polynucleotides by polynucleotide phosphorylase. J. Biol.Chem. 250: 508-514, 1975.

2. Letendre, C. H., and Singer, M.F.: Further characterization of the polynucleotide phosphorylase of Micrococcus luteus. Nucleic Acids Res. 2: 149-163, 1975.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 333-13 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The biochemical basis for defective maturation in myelogenous leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Evans	Research Chemist	LB NCI
OTHERS:	E. A. Peterson	Research Chemist, Head Protein Chemistry Section	LB NCI
	M. G. Mage	Senior Dental Surgeon	LB NCI
	S. Wilson	Biologist	LB NCI
	R. Rao	Visiting Fellow	LB NCI

COOPERATING UNITS (if any)

Charles K. Grieshaber, Staff Fellow Laboratory of Biology, DCBD, NCI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Protein Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

The main thrust of this work is to develop biochemical methods for the early diagnosis of granulocytic leukemia and methods for inducing leukemic cells to develop some or all of their functional properties as a means of partially or completely restroing host defense mechanisms in leukemia patients. Work is first aimed at identifying the main molecular constituents and metabolic processes that distinguish normal mature granulocytes from their immature precursors and establishing which of the many steps involved in granulocyte maturation are regulated by factors present in body fluids and/or by cell-mediated factors. The results will be compared with those obtained from similar studies on leukemic cells at corresponding stages of maturity in order to determine the nature and potential reversibility of the arrested maturation steps. Biochemical analyses of molecular constituents are carried out on mature and immature granulocytes isolated from blood and bone marrow by velocity sedimentation and density centrifugation procedures developed in this laboratory. The effects of external cell regulators on granulocyte differentiation, as measured by changes in the synthesis of specific cellular components, are studied in a chemically defined culture system previously developed in this laboratory.

PHS-6040
(12-75)

Major Findings: Development of Biochemical Assays for Regulators of Granulocyte Proliferation and Differentiation in the Bone Marrow. We are exploring the possibility of using the response of the bone marrow to inflammatory stimuli as a model for studies of the regulation of granulocyte production. Inflammatory exudates were induced in guinea pigs by intraperitoneal injection of a sterile solution of glycogen in saline. At various time intervals after injection absolute and differential counts of the cells in the granulocyte compartmemt in the marrow of the hind limb were determined. The results showed that within 12 hours after injection 81% of the mature non-proliferating granulocytes had been released into the circulation. In the proliferating cell compartment, the blast plus promyelocyte count decreased by 42%, whereas myelocytes showed a slight increase during this period. After 12 hours, the mature cell and blast plus promyelocyte counts increased steadily, reaching normal levels by 36 hours. The myelocyte count showed only minor changes during this recovery phase of the inflammatory response.

We have shown previously that one of the striking biochemical changes that occurs during granulocyte differentiation is the appearance of several glycoproteins specifically associated with the characteristic cytoplasmic granules of neutrophil granulocytes. Previous methods for extracting these proteins from granules involved the use of hot SDS solutions which cause denaturation of these proteins. Further characterization of these proteins by chromatographic, immunochemical and enzymatic methods, however, require the development of less drastic non-denaturing techniques for extraction and solubilization. We have found that up to 50% of the granule proteins can be extracted with either 0.1% cetyltrimethylammonium bromide (CETAB) or 1.0% Triton-X-100 at 4°. Increasing the CETAB concentration up to 1.0% yields another 30% of the granule protein whereas higher concentrations of triton-X-100 produced no further increase in extractable protein. The granulocyte proteins in these extracts all migrate as cations, with good resolution of the major proteins, during polyacrylamide gel electrophoresis at pH 4.7. Assays for granule enzymes such as myeloperoxidase and alkaline phosphatase indicate that such enzymes are not inhibited by the extraction conditions. Incubation of granulocyte precursors in Eagle's medium containing leucine-^{14}C resulted in incorporation of label into the protein extracts that was linear up to 4 hours, and all of the major granule proteins in the gels were labelled.

2. Differences in the Content and Synthesis of Polyamines in Immature and Mature Granulocytes. Using cell separation techniques previously developed in our laboratory we have isolated highly enriched fractions of immature and mature granulocytes from guinea pig bone marrow and measured their content of putrescine, spermidine, and spermine. The two cell preparations differed chiefly in their spermidine content, which was 3-4 times greater in the immature than in in the mature cell fraction. Ornithine decarboxylase (ODC), the first enzyme in the pathway of polyamine synthesis, was about 10 times more active in immature than in mature cells. The rate of incorpo-

ration of ornithine-^{14}C into putrescine and spermidine of intact cells *in vitro* was about 5-6 times greater in the immature than in the mature cells. Both immature and mature granulocytes failed to convert arginine-^{14}C to polyamines. This conversion would require the coordinate action of arginase and ODC. We found previously, however, that these enzymes develop asynchronously during granulocyte development.

Significance to Cancer Research: (Objective 2, Approach 3) Understanding of normal and leukemic granulocyte maturation depends upon clarification of the molecular processes whereby the cell controls the appearance and disappearance of specific components. Such information has never been obtained at the biochemical level in a systematic fashion similar to that achieved in morphological studies of normal and leukemic granulocytes. Any biochemical differences in the maturation processes observed between normal and leukemic leukocytes will be exploited for purposes of developing techniques, presently unavailable, for the early diagnosis of leukemia and for improved treatment, especially with regard to the possibility of reversing the arrested maturation and decreased phagocytic activity of leukocytes from myelogenous leukemia.

Proposed Course of Research: The rate of production of granulocytic leukocytes in the bone marrow is regulated by a feedback control mechanism which responds to the rate of destruction of these cells in the peripheral tissues of the body. Feedback information could be of a positive (stimulator) or negative (inhibitor) type transmitted by humoral and/or cellular factors, but at present the nature of these factors is unknown. We are using the response of guinea pig bone marrow granulocyte precursors to inflammatory stimuli as a model for studying the nature of the feedback factors involved in the regulation of granulocyte production. The humoral hypothesis for regulation will be examined by studying the effect of sera from normal and inflammatory guinea pigs on the synthesis of granule-specific granulocyte precursor proteins by intact cells *in vitro*. This approach is analogous to the use of hemoglobin as a marker for erythrocyte production in assays for erythropoietin. Unlike erythrocytes, which contain only one major specific protein, granulocytes contain many potential marker proteins in their granules and it is conceivable that humoral regulators might affect the synthesis of some granule proteins more than others. In our preliminary studies, therefore, we plan to use the radioactive assay of granule proteins in slices of electrophoretic gels described above as a screening technique for humoral regulators in sera. If such regulators are detected by this method, then more specific immunological assays for the specific proteins affected by these sera will be developed.

Studies are underway to further clarify the role of polyamines in granulocyte formation in the bone marrow. Since the conversion of cells from the resting to the proliferating state is associated with a dramatic increase in ODC activity and subsequent polyamine sythesis, one might expect that bone marrow granulocyte precursors would be similarly affected if granulocyte proliferation were stimulated by inflammatory stimuli. Thus changes in ODC activity and/or polyamine synthesis might serve as useful indicators in detecting hormonal and/or cellular feedback regulators of granulocyte production. Appropriate experiments using highly enriched preparations of bone marrow granulocytes will be carried out to test these hypotheses.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 375-14 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Homogeneity and structure of proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. A. Peterson	Head, Protein Chemistry Section	LB NCI
OTHER:	A. R. Torres	Research Associate	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Methods for the fractionation and analysis of proteins are developed and applied to the purification of specific proteins for the study of their function and structure. Current efforts are directed toward the development of displacement chromatography for the fractionation of macromolecules and particles of biological interest, employing polyanions and polycations differing in molecular size and charge density as displacers.

Project Description:

Objectives: To develop methods for the separation and analysis of proteins that will overcome present limitations and to apply these methods to the purification of specific proteins for the study of their function and structure.

Methods Employed: A variety of polyanions and polycations differing in molecular size, charge density, and chemical composition are prepared by appropriate reactions. Most of these are ionic derivatives of low molecular weight dextrans but other types are being explored. They are evaluated as displacing agents, using test protein mixtures, on hydrophilic ion exchangers. Effluent fractions are examined by electrophoresis in slabs of polyacrylamide gel and by appropriate assay.

Major Findings: The preparation and use of carboxymethyldextran mixtures that provide smoothly rising affinity continuums for ion-exchange displacement chromatography of proteins were mentioned in last year's report. In the period covered by the present report, attention has been given to the dilemma posed by the technical disadvantages of using adsorbent columns large enough to accommodate the sample proteins plus the spacing displacers simultaneously and the opposing disadvantages of using much smaller columns. These problems are mitigated, to a considerable extent, by the use of a preliminary column of DEAE-cellulose to develop the displacement train of CM-dextrans before it enters a small DEAE-agarose column into which the protein sample has previously been introduced. (Although the adsorption capacity for proteins is good, its capacity for adsorbing 10,000 M. W. CM-dextrans is much less than that of DEAE-cellulose.) Another approach to the problem of overloading the small columns with a displacer continuum is that of preparing the CM-dextran continuum in sections to be pumped successively into the protein-separating column. Thus, in effect, a substantial portion of the function of the column is achieved and preserved in the preparation of the displacers, so that those pumped into the column at any one time represent a much narrower range of affinities. This method of operation also has the effect of greatly diminishing the concentration of displaced counter ions, since the displacement of these ions is spread over a much larger volume.

The preparation of such "sectioned affinity continuums" is facilitated by the use of a programmed temperature rise that essentially straightens out the reaction curve, which otherwise would flatten out and make necessary progressively longer reaction intervals for successive sections. A novel, very simple device has been constructed that permits preselected volumes of ten sections to be pumped sequentially into the column automatically.

Significance to Cancer Research: (Objective 2, Approach 3). Displacement chromatography of proteins, nucleic acids, etc., promises to be of value at any scale of operation and therefore has significance to all research that involves the isolation of such substances. The displacers, unlike salt, do not interfere with electrophoresis, and proteins fractions obtained by displacement can be directly subjected to electrophoresis or rechromatography without dialysis or concentration. Thus the examination of complex mixtures by a combination of chromatography and electrophoresis on a semi-micro scale is greatly expedited. The simplicity of operation also facilitates automation, so that the two-dimensional analysis of large numbers of fractions becomes feasible.

Moreover, since migration is induced by displacers under conditions of salt and pH in which the migrating molecules are otherwise strongly adsorbed, the possibility arises of chromatographing substances that are "irreversibly" adsorbed in conventional procedures. Also, theoretical considerations strongly suggest that many of the enzymes that become labile when purified can be protected from inactivation or loss by substances such as the CM-dextrans if the metal-sequestering properties of the system are taken into account.

Proposed Course of Research: The method, as it develops, will be applied to the isolation of a serum factor (or factors) that mediates bone marrow response to experimentally induced inflammation in the guinea pig. Its possible application in the fractionation of cell populations and subcellular organelles will be explored, using appropriate adsorbent matrices. Also, a stabilizing effect of the CM-dextrans will be looked for, using enzymes that have previously been difficult to purify because of their high lability.

Publications: None*

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5202-09 LB

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Isolation, fractionation, and charcterization of native nucleoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	O. Wesley McBride	Medical Officer, Res.	LB NCI
OTHER:	Raghbir S. Athwal	Visiting Fellow	LB NCI
	Miles C. Otey	Research Chemist	LB NCI
	Elbert A. Peterson	Chief, Protein Chemisty Section	LB NCI

COOPERATING UNITS (if any)

(1) Laboratory of Pathology, DCBD, NCI
(2) Division of Med Genetics, Dept. of Med, Johns Hopkins U. School of Medicine

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop methods for gene mapping in eukaryotic cells. Gene transfer is detected by isolation of colonies of biologically transformed cells in selective medium after incubation of isolated metaphase chromosomes with mutant recipient cells deficient in HPRT or TK. The gene product is further characterized as chromosomal donor species. Cotransfer of two closely linked genes, TK and galactokinase, occurs by this procedure. The method currently may be most useful for determining close gene linkages.

PHS-6040

Objectives: The determination of the role of nucleic acid-bound proteins in the regulation of the transcription and translation of genetic information, and the biological transformation of cells with isolated and fractionated mammalian metaphase chromosomes as a means of genetic mapping.

Methods Employed: Tissue culture procedures involving propagation of mammalian cells in monolayer and suspension cultures, plating in selective media, cloning, labelling with radioisotopes, selection of cell mutants, and metaphase arrest for chromosome isolation. Metaphase chromosome isolation and purification by isopycnic and velocity sedimentation (including unit gravity procedures), and evaluation of chromosomal DNA molecular weight by zonal density gradient sedimentation. Gene transfer to mammalian cells via uptake of purified metaphase chromosomes and isolation of biologically transformed colonies in selective media. Isolation, quantitation, and characterization of gene products by chromatography, electrophoresis, isoelectric focusing, and immunochemical procedures. Karyotic analysis of cells by fluorescense, phase- and bright-field photomicroscopy. Fixation and staining of cells and chromosomes with fluorochromes for flow microfluorometric analysis of DNA content. Isolation of DNA and nucleic acid hybridization techniques.

Major Findings: A human gene for hypoxanthine phosphoribosyl transferase (hprt) has been serially transferred to mouse cells and then to Ch. hamster cells via the intermediate process of two chromosome isolations and uptake of chromosomes by mutant recipient cells. The HPRT gene product of three independent colonies (representing products from two different donor lines) has been identified as human species by gel electrophoresis. Only Chinese hamster chromosomes were found on karyotypic analysis of cells from these same three colonies. These results indicate that genes transferred by the process of chromosome uptake are probably integrated into the chromosomal DNA of the recipient cell.

Colonies have been isolated in selective (HAT) medium after incubation of TK-deficient B_{82} mouse cells with metaphase chromosomes isolated from wild type Chinese hamster fibroblasts. Efforts to distinguish Chinese hamster from mouse species of thymidine kinase by a large number of physical separation procedures have been unsuccessful. However, these two species can be distinguished by differing thermostability. Thus, the TK in 12 independent transformed clones has been identified as chromosomal donor species. These 12 colonies exhibit a wide variety of stability of the transformed phenotype. Two of these same clones (2/10 examined) also express the chromosomal donor species of galactokinase, indicating that cotransfer of closely linked genes occurs with an incidence of about 20%. Back-selection of both colonies in BUdR for loss of TK also resulted in the coordinate loss of the hamster, but not mouse, species of galactokinase, thus providing proof that cotransfer of both genes did occur.

DNA has been isolated from the B82 recipient line and both transformed clonal lines containing both TK and Gal K in the transgenome. A radiochemical-labeled DNA probe, containing repetitive DNA sequences found in the transformant but not present in the parental recipient B82 line, has been prepared by the collaborating group at Johns Hopkins. The reassociation kinetics of this probe were assessed, employing driver DNA of various types, including that from B82 cells, the same transformant clone, and hamster chromosomal donor species. The results indicate there is a fraction of radiochemical-labeled repetitive DNA which is homologous with hamster, but not with mouse, DNA. Preliminary estimates indicate this represents about 0.04% of the total DNA in the transformed cell. This implies that the transgenome represents no more than about 1/20 of a chromosome (about 4×10^6 nucleotide base pairs).

Metaphase chromosomes from Ch. hamster cells were successfully evaluated by FMF by Ms. B. J. Fowlkes (Lab. of Pathology), employing procedures virtually identical to those reported recently by Gray et al. from the Livermore Laboratories. This now provides a rapid and sensitive tool for evaluating chromosome fractionation techniques.

Clones have been isolated in selective medium following the incubation of Ch. hamster metaphase chromosomes with HPRT deficient human cells. Analysis of extracts for the species of origin of HPRT, G6PD, and phos phoglycerate kinase (X-linked markers) is now in progress. Five of these independent transformed clonal lines have been separately fused with a double mutant mouse line and a number of independent hybrid cell lines have been isolated in a selective medium. Analysis of segregants is in progress to determine concordance between the retention of a specific human chromosome with retention of the hprt gene, thereby indicating the integration site of the transgenome.

Proposed Course of Research

Studies are in progress to determine the size (and variation in size) of the transgenome introduced by chromosome mediated gene transfer. Molecular DNA hybridization and FMF provide the main tools for this investigation.

The possible integration site(s) of the transgenome will be evaluated. Molecular hybridization will provide evidence as to whether the transgenome is covalently linked to mouse recipient DNA. Analysis of segregants of hybrid cells obtained by fusing mouse cells to human cells containing hamster hprt in a transgenome should identify the human chromosome, (or chromosomes) containing the integrated transgenome.

Metaphase chromosomes will be isolated from mouse cells containing the transferred hamster TK gene alone and from cells containing the transferred TK and galactokinase. These chromosomes will be incubated

with TK- human cells. If the integration site for the transgenome is a homologous region of the DNA of a recipient cell (as occurs in meiotic crossing-over), the resultant clonal lines may express both the mouse and human species of galactokinase or all three species of this enzyme. This result would obviously strongly support a model of this type.

Recent studies in another laboratory suggest that intraspecies chromosome mediated gene transfer may occur with much higher frequency than interspecies transfer and it may permit transfer of an intact chromosome. Mus caroli and mus mus are closely related species and interspecies hybrids can be formed in vivo. However, the 3 X-linked markers HPRT, G6PD, and PGK are all electrophoretically distinguishable between these 2 species. Hence metaphase chromosomes isolated from mus caroli will be incubated with HPRT-deficient mus mus cells (A_9) and resultant clones will be analyzed for transfer of an intact X-chromosome.

Significance to Cancer Research: (Objective 2, Approach 3) Metaphase chromosome isolation and fractionation provides the most promising approach for the fractionation of mammalian genes and can provide material for the investigation of control elements associated with specific sets of genes. The biological expression of these chromosomes introduced into recipient cells provides a method for mapping of mammalian genes, investigating differences between genetic elements in normal and neoplastic cells, and looking for sites of integration of oncogenic viruses into the mammalian genome. Demonstration of cotransfer of closely linked genes indicates this may be a valuable method for mapping these genes (which cannot be dissociated by other methods). These cell lines containing 2 markers in the transgenome appear to be extremely valuable tools for investigating gene modulation, sites of integration, and obtaining relatively short regions of repetitive DNA adjacent to structural genes (and possibly the structural genes also).

Publications:

1. Burch, J. B., and McBride, O. W.: Human Gene Expression in Rodent Cells After Uptake of Isolated Metaphase Chromosomes: Proc. Nat. Acad. Sci. USA 72: 1797-1801, 1975.
2. McBride, O. W.: Permanent Transfer of Genes to Eukaryotic Cells by Uptake of Metaphase Chromosomes in "Symposium on Somatic Cell Genetics" In Vitro 14:--------1976.
3. Ruddle, F. H., and McBride, O. W.: Gene Transfer in Somatic Cell Genetic Systems in "The Molecular Biology of the Mammalian Genetic Apparatus; Its Relationship to Cancer, Aging, and Medical Genetics." P. T'so, ed. The Assoc. Scientific Publishers, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 5203-08 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of immunochemical and physical methods for the purification and characterization of immunocytes and their subcellular components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. G. Mage	Dental Director	LB NCI
OTHER:	T. L. Rothstein	Staff Fellow	LB NCI
	L. L. McHugh	Biologist	LB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Protein Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the development of cell separation methods for the specific isolation of immune cells, particularly for antigen-reactive cells involved in cellular immune reactions, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules. Antigen-reactive lymphocytes (ARC) are obtained from the spleens of normal mice and from mice immunized with allogeneic tumor cells and normal cells. Spleen cell suspensions containing ARC are tested for binding to the cell surface antigens of target cells attached to insoluble supports. The nonadherent cells are tested for depletion of cytotoxic effector activity by measurement of ^{51}Cr release from target cells. Craft-versus-host (GVH) activity is measured by the Simonsen spleen weight assay in neonatal mice.

Objectives: The goal of this project is the development of methods for the specific isolation of immune cells, particularly for antigen-reactive cells involved in cellular immune reaction to alloantigens, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules.

Methods Employed: Antigen-reactive cells (ARC) are obtained from the spleens of normal mice or from mice immunized with allogeneic tumor cells and normal cells. Spleen cell suspensions containing ARC are incubated on foam columns or on petri plates coated with allogeneic or syngeneic target cells. Graft-versus-host (GVH) activity of non-adherent spleen cells is measured by the Simonsen spleen weight assay in neonatal F_1 mice. Cytotoxic activity is measured by ^{51}Cr release from target cells.

Major Findings

We have previously found that allogeneic spleen cells, but not embryonic fibroblasts, can serve as target cells for the binding of GVH-ARC, as evidenced by the specific depletion of about 50% of the GVH activity from the cell population that does not adhere to monolayers of target cells.

Following up on this finding, we have separated the spleen cells into those with surface immunoglobulin (B cells) and those without surface immunoglobulin (mostly T cells). We developed a simplified preparative method for doing this separation, based on the incubation of spleen cell suspensions in petri plates precoated with specifically purified anti-immunoglobulin.

The separated B cells and T cells, when tested as target cells, could both bind GVH-ARC, but the extent of the depletion of GVH activity was still only 50%. In other experiments, the extent of the separation remained the same whether target spleen cells were from homozygous C57BL/6 mice or from heterozygous [C57 Bl/6 x BALB/c] F_1 mice, or whether or not the spleen cells were treated with neuraminidase.

We are currently attempting to delineate further the basis for the heterogeneity of GVH-ARC with respect to in-vitro binding to allogeneic monolayers. We are also examining the mechanism of *in vitro* lysis of allogeneic cells by immune T cells, and working on a general method of cell separation, based on the binding of antibody-coated cells to anti-immunoglobulin-coated surfaces.

Significance to Cancer Research: (Objective 2, Approach 1) These studies on the specific separation of antigen-reactive cells relate to the mechanisms of cell-mediated tumor rejection. Knowledge of the antigen-binding characteristics of immune cells may help in development of specific purifications of immune lymphocytes with antitumor reactivities and may aid in understanding the variation in tumor susceptibility of human beings and make possible control of immune mechanisms of tumor surveillance and rejection.

The GVH reaction is an iatrogenic complication of tumor therapy regimens that include bone-marrow transplantation. A specific separation of GVH-antigen reactive cells might aid in avoiding this complication.

Proposed Course of Research: As an aid to the development of general methods for the specific isolation of antigen-reactive lymphocytes, we plan to further study the antigen-binding characteristics of immune T-cells, particularly with respect to target-cell antigen specificity, the conditions needed for antigen binding by ARC, and the conditions needed for the stable binding of lymphocytes to insoluble substrates. We are currently attempting to delineate further the basis for the heterogeneity of GVH-ARC with respect to in-vitro binding to allogeneic monolayers. We are also examining the mechanism of *in vitro* lysis of allogeneic cells by immune T cells, and working on a general method of cells to anti-immunoglobulin-coated surfaces.

Publications:

1) Specific Partial Depletion of Graft-Vs-Host Activity By Incubation and Centrifugation of Mouse Spleen Cells on Allogeneic Spleen Cell Monolayers. M. G. Mage, L. L. McHugh, J. Immunol., 115:911 (1975).

2) Separation of Graft vs Host Antigen Reactive Cells *in Vitro*. M. G. Mage, L. L. McHugh, T. L. Rothstein, Transpl. Proc. *in press* 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 5230-03 LB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biochemical Properties of Neoplastic Cell Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. J. Marciani	Research Chemist	LB	NCI
OTHERS:	E. A. Peterson	Research Chemist, Head, Protein Chemistry Section	LB	NCI
	E. B. Thompson	Medical Director, Head, Biochemistry of Gene Expression Section	LB	NCI
	J. P. Bader	Senior Research Microbiologist	DCCP	NCI
	T. Okazaki	Visiting Fellow	DCCP	NCI

COOPERATING UNITS (if any)
Chemistry Branch, DCCP, NCI

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The modifications of membrane components, mainly proteins, that occur in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus are studied in order to ascertain the role of such modifications in alterations of cell properties that are expressed at or mediated by the cell surface. Cell membranes are isolated and their composition determined by electrophoretic and chromatographic procedures. Membrane surface properties are investigated by the use of lectins and labeling with surface-selective techniques.

PHS-6040
(12-75)

Project Description:

Objectives:

The study of membrane components, mainly proteins, that can be modulated in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus, or in somatic cell hybrids between the malignant cells mouse LB82 and rat hepatome HTC-1. The study of the role of these proteins in properties of the cells that are mediated by, or expressed at the cell surface.

Methods Employed:

Isolation of cell membranes in an aqueous two-phase system. Analysis of the membrane composition by electrophoretic and chromatographic techniques. Study of the cell surface topography by use of enzymic labeling with ^{125}I or ^{3}H, combined with electrophoresis. Isolation of proteins expressed in the cell membrane of somatic cell hybrids and their parental cell lines for comparison by peptide mapping, in order to determine their origin and control of expression.

Major Findings:

Studies during the past months were concerned primarily with plasma membranes from cell hybrids between mouse L cells and rat hepatoma, HTC, isolated by Dr. E. B. Thompson, and chick cells infected with a thermosensitive mutant of Rous sarcoma virus, isolated by Dr. J. P. Bader.

(1) Cell membrane from mouse L cells (L-B82), rat hepatoma (HTC-H1), and three clones of their somatic cell hybrids (07, V4a, V5) showing different degrees of density-dependent inhibition of growth were analyzed by polyacrylamide gel electrophoresis. The membrane polypeptides of the hybrid clones were all similar and all showed noteworthy differences from one or both parents. The cell hybrids showed, for example, higher proportions of polypeptides of molecular weight 56,000 and 45,000 than their parents or their normal counterparts, mouse primary embryo and rat liver cells. The major glycoprotein from cell hybrids seemed to be identical to that of rat liver or rat hepatoma cells and different from that of L cells.

One hybrid showed density-dependent inhibition of growth; the other two, like both parents, did not. All hybrids and both parents produced tumors in nude mice, although tumor production by the hybrids was delayed. The parental cells and hybrids were compared for their content of a large external protein (M.W. 240,000) iodinated by lactoperoxidase-catalyzed reaction. This protein was virtually missing in the parents but present at high levels in all their hybrid clones. Thus there was a lack of correlation between the presence of this protein, growth control in vitro, and tumorigenicity. Furthermore, a similar lack of correlation was seen in studies of the agglutination of these cells by concanavalin A. It is postulated that the factors controlling these membrane alterations are independent of density-dependent inhibition of growth and of those controlling the expression of malignancy.

(2) Chick Embryo cells infected with a mutant (Ta) of the Bryan high titer strain of Rous sarcoma virus (RSV-BH) developed hemadsorbing activity at the nonpermissive temperature for transformation, 41°C. Incubation of infected cells at 37°C, the permissive temperature for transformation, resulted in loss of hemadsorbing activity. The changes were reversible and did not depend on RNA or protein synthesis. Treatment of cells with phospholipase c or incorporation of 2-deoxy-D-glucose into the cells resulted in inhibition of the hemadsorption. The results suggest that hemadsorbing activity may be due to properties of the lipid fraction in the cell membrane.

Significance to Cancer Research: (Objective 3, Approach 5) The fact that the sociology of cells is mediated through their cell surfaces makes the study of cell membranes important for understanding the lack of contact inhibition, the changes in permeability, and the capacity for invading normal tissue that are characteristic of neoplastic cells.

Proposed Course of Research: We are planning to study the changes in glycoproteins at the plasma membrane level as a function of temperature shift in cells infected with the thermosensitive mutant of Rous sarcoma virus. These studies will involve the isolation of lectin receptors and determination of their locations in the cell membrane, changes in membrane fluidity (using fluorescent probes), and changes related to temperature shifts. These several changes will be correlated with the altered properties of cell membranes upon transformation.

Using somatic cell hybrids expressing different degrees of density-dependent inhibition of growth, we hope to correlate some characteristics such as expression of new proteins with growth properties and tumorigenicity.

Publications: Marciani, Dante J. and Bader, John P.: Polypeptide composition of cell membranes from chick embryo fibroblasts transformed by Rous sarcoma virus. Biochim. Biophys. Acta (1975) 401, 386-398.

SUMMARY STATEMENT
LABORATORY OF MOLECULAR BIOLOGY
DCBD, NCI
July 1, 1975 - June 30, 1976

The Laboratory of Molecular Biology has been expanded by the addition of the space and personnel from the Laboratory of Biology upon the retirement of Dr. Heston. A new section on Gene Regulation has been established and Dr. deCrombrugghe will be appointed as Chief of this section. The personnel from the Laboratory of Biology have been transferred into the Section on Gene Regulation.

The research effort of the Laboratory is divided into four broad areas of interest:

1. The biochemical basis of malignant transformation with particular emphasis on the role of Cyclic AMP and cell surface protein (CSP) in this process.

2. Regulation of gene activity with particular emphasis on the identification of molecules that activate genes and the determination of the structure of the regulatory regions of genes.

3. Investigation of how the thyroid cell forms, stores and releases its hormone and the elucidation of factors that control its growth, differentiation and transformation into a malignant cell.

4. Investigations on mammary tumor virus and other oncornaviruses and elucidation of how these viruses are synthesized and how they change normal cells into cancer cells.

Part I. The Biochemical Basis of Malignant Transformation.

When propagated in tissue culture malignant cells can be distinguished from normal cells by their altered morphology, decreased adhesiveness, altered motility, increased agglutinability by plant lectins and their ability to grow in the absence of certain serum growth factors. A major effort has been devoted towards understanding the biochemical basis of these differences. Previous studies have implicated cyclic AMP as one factor responsible for alterations in cell shape, adhesiveness and other properties.

Low cyclic AMP levels are due to low activity of adenylate cyclase; often adenylate cyclase is altered so that it no longer responds to hormones. P. Davies has investigated the adenylate cyclase of a variety of transformed cell lines whose adenylate cyclase was previously shown by W. Anderson to be unresponsive to prostaglandin E_1. P. Davies has found that the unresponsiveness is due to loss of the prostaglandin receptor. Insulin is a growth factor for some fibroblastic cells and controls cyclic AMP levels. P. Thomopoulos measured the insulin binding capacity of five lines of

transformed 3T3 cells and found that all five transformed cell lines had greatly diminished insulin binding capacities and this was associated with a decreased serum requirement for growth. Thus, it appears that transformed cells lose their ability to bind serum substances that are involved in growth control. This finding suggests that a hormone receptor might inhibit growth when unoccupied and, when occupied, change to a form that stimulates growth.

W. Anderson has continued his investigations on adenylate cyclase in cultured cells and found that a number of unsaturated fatty acids inhibit adenylate cyclase activity. Saturated fatty acids are without effect, although they can activate the guanylate cyclase of these cells. Anderson has also found evidence of a high molecular weight factor in serum which selectively inhibits the stimulation of adenylate cyclase by hormones and GTP. He is purifying this material and evaluating its possible role in growth control.

Cyclic GMP - previous work from other laboratories suggested that cyclic GMP might function to stimulate the abnormal growth of transformed cells. We have been unable to confirm that cyclic GMP levels are elevated in transformed cells and instead find that cyclic GMP levels are often low in such cells. This appears to be due to diminished guanylate cyclase activity. To understand the regulation of guanylate cyclase activity, D. Wallach isolated a fraction from serum that activated guanylate cyclase and identified the active material to be a mixture of fatty acids. Whether serum fatty acids control guanylate cyclase activity in vivo is currently under investigation.

M. Willingham is studying the surface microstructure of cells. Last year he presented results indicating that the basis for enhanced agglutinability of transformed cells by lectins was their increased content of microvilli. He has now examined 15 different normal and transformed cell lines and they all fit into the expected pattern. M. Willingham is also investigating the localization myosin and other contractile proteins in cells.

K. Olden has continued studies on the myosin associated with the plasma membrane employing lactoperoxidase catalyzed iodination of intact cells. His results indicate that myosin is probably a transmembranal protein, and is probably associated with a glycoprotein in the plasma membrane of the cell. Myosin maybe held in the membrane by this protein.

J. Pouyssegur has isolated mutant 3T3 cells that are decreased in their adhesiveness to substratum. Morphologically these mutants resemble transformed cells, but they have the growth properties of normal cells. Thus, one can separate round shape and low adhesion from altered growth control. The mutant cells are defective in glycoprotein synthesis and synthesize decreased amounts of most cell surface glycoproteins. Apparently the decreased adhesiveness is due to the decreased synthesis of one or more membrane proteins involved in the adhesive process.

K. Yamada has isolated the major cell surface glycoprotein (CSP) from chick embryo fibroblasts. He has been able to add this protein back to transformed cells that have diminished amounts of the protein and restore

normal shape, adhesion and contact inhibition of movement to transformed cells. Thus CSP has many of the same effects as cylcic AMP. However, the two molecules work independently. Neither controls the synthesis of the other and CSP does not effect cell growth whereas cyclic AMP does. Cyclic AMP does not effect the shape of transformed chick cells, whereas CSP does. Thus, in the last few years we have identified two molecules, cyclic AMP and CSP which have important roles in determining the phenotype of malignant transformation.

G. Johnson is studying the nature of altered sugar metabolism in transformation. He employs a cell line which grows only on glucose but can be adapted to grow on a variety of other sugars if exposed to a single other sugar for a few days. For example, after a few days exposure to galactose, the cell line will grow on galactose and many other sugars.

Part II. Control of Gene Activity and the Structure of Regulatory Loci.

I. Pastan and D. Schlesinger (Harvard University) have continued studies on the primary structure of the cyclic AMP receptor protein of E. coli. These results may lead to an understanding of how this protein interacts with DNA. The protein is very difficult to sequence because of our inability to obtain fragments near the amino terminus of the molecule. A variety of different approaches have failed.

B. deCrombrugghe, R. Musso, and R. Dilauro have determined the sequence of the operator-promoter region of the galactose operon. They have tentatively identified the portion of the sequence to which CRP binds and have begun to analyze mutants in the operator region to determine the molecular basis of repressor action. They have also found that the site of initiation of transcription determined by cyclic AMP and CRP may differ by about five residues from the site where transcription begins in the absence of cyclic AMP.

B. Howard has investigated the mechanism of action of termination factor rho and found that the intrinsic ATPase activity of rho is required to carry out rho mediated termination of transcription.

M. Rosenberg has investigated the primary nucleic acid structure at termination points of different genes. He finds that the nucleic acid structure of 3' terminal regions from λ and λ gal transcripts possess similar degrees of 3' terminal sequence heterogeneity which mainly consist of the addition of adenylate residues. Rho factor enhanced termination results in a definite structural change in the nucleotide sequence with which an RNA molecule terminates.

S. Adhya, M. Gottesman, D. Court, and A. Das have investigated the genetic basis of the control of gal operon expression as well as λ expression. They have isolated an E. coli mutant defective in termination factor rho. Thus, they can assess the precise role of rho in termination of E. coli genes. Current evidence suggests that rho is the only termination factor in E. coli. The termination activity of rho can be overcome by a gene product of both phage λ and phage P22. The biochemical basis of this anti-termination effect is now being investigated.

M. Shulman using techniques developed to analyze the structure of λ DNA is initiating studies on the primary structure of the immunoglobulin genes of immunoglobulin producing cells. He is using a bacteriophage model (T7) to make molecules analogous in structure to those expected for hybrids of immunoglobulin messenger RNA and germ line DNA.

Part III. Control of Activity of Thyroid Cells.

S. Wollman and J. Zeligs are investigating the mechanism by which colloid is taken from the follicular lumen into the cell. They find the thyroid cell can also phagocytoze red blood cells and that pseudopod formation may be induced by the presence of particulate objects such as red cells or pseudopods on adjacent cells.

S. Wollman, F. Ambesi and H. Coon have been able to grow thyroid tumor cells in tissue culture without any special modification of the medium, whereas normal thyroid ipithelial cells require medium conditioned by prior exposure to certain fibroblasts treated with Bt_2 cAMP. J. Zeligs has been examining the ultrastructural changes in cytoplasmic organelles during mitosis.

Part IV. Mammary Tumor Virus and Related Studies.

G. Smith is investigating the intracytoplasmic A particles found in most mammary tumors and their relationship to purified mouse mammary tumor virus. He has isolated this virus from cells and characterized its protein and nucleic acid composition. It remains to be clarified whether this virus is a precursor of mouse mammary tumor virus or is along a developmental side path. The relationship of the virus to the development of mammary tumors is also under study. G. Vlahakis is investigating the genetics of transmission of mouse mammary tumor virus in different strains of mice.

K. Stromberg has undertaken a study to try and rescue an oncornavirus from the human placental cells.

H. Hoffman is employing genetic techniques in mice to study gene regulation of protein turnover and its relationship to tumorigenesis. His studies on catalase have revealed that the alterations in catalase in mutant mice is due to some type of post-translational modification of the molecule. Other studies concern the inheritence of the expression of MTV group specific antigens. C. Grieshaber has initiated studies on polyamine metabolism in differentiating cells; polyamine synthesis changes markedly during differentiation.

Addendum. Projects "Control of Expression of the Galactose Operon in E. coli K12, Bacteriophage λ and P22" by Sankar L. Adhya, Max E. Gottesman and Donald L. Court, Serial No. ZO1 CBD 8006-04 LMBGY, and "Factors that Regulate Cyclic AMP Levels in Cultured Cells" by George S. Johnson, Serial No. ZO1 CBD 8009-02 LMBGY have been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 8001-06 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ira Pastan, Chief, Laboratory of Molecular Biology, DCBD, NCI

Leon Moore, Guest Worker, Laboratory of Molecular Biology, DCBD, NCI
David Wallach, Visiting Fellow, Laboratory of Molecular Biology, DCBD, NCI
Peter Davies, Surgeon, Laboratory of Molecular Biology, DCBD, NCI
Robert Shiu, Guest Worker, Laboratory of Molecular Biology, DCBD, NCI

Pierre Thomopoulos, National Institute Arthritis, Metabolism and Digestive Diseases.

COOPERATING UNITS (if any)

National Institute Arthritis, Metabolism and Digestive Diseases

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	5	2

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclic AMP and Cyclic GMP levels tend to be low in transformed cells, due to diminished adenylate and guanylate cyclase activities. Transformed cells bind diminished amounts of regulatory molecules such as insulin and prostaglandin E1.

Serial No. ZO1 CBD 8001-06 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed and Normal Fibroblasts

Previous Serial Number: ZO1 CBD 8001-05 LMBGY

Principal Investigator: Ira Pastan

Other Investigators: L. Moore, David Wallach, Peter Davies, and Robert Shiu

Cooperation Units: NIAMDD

Man Years: July 1, 1975 through June 30, 1976

Total:	7
Professional:	5
Others:	2

Project Description:

Objectives: To understand the role of cyclic AMP in malignant transformation and how cyclic AMP regulates the growth, morphology, and other properties of cultured fibroblastic cells.

Methods Employed: Cell culture and standard biochemical analyses of the enzymes involved in cyclic AMP metabolism and the synthesis of macromolecules.

Major Findings: Cyclic Nucleotide Assays-Because other investigators have reported that cyclic GMP levels are elevated in growing cells we have employed a cyclic GMP assay to measure cyclic GMP. We find that the levels of cyclic GMP are not elevated in growing cells and often fall when non-growing cells are stimulated to grow by serum addition. Cyclic GMP and cyclic AMP levels often change together.

Adenylate cyclase (A.C.) A variety of transformed cell lines have been identified in which the response of A.C. to PGE_1 is abolished. P. Davies has studied the binding of PGE_1 to membranes from these cells and finds that the loss of the ability of A.C. to be activated by PGE_1 is associated with diminished PGE_1 binding. If a coupling factor were needed for PGE_1 to activated A.C., cells with high binding but a PGE_1 insensitive A.C. might have been found. None were.

Previously we noted that one or more factors present in the cytoplasm of cultured cells had the capacity to activate A.C. in the presence of PGE_1. R. Shiu has shown these factors are proteins to which GMP or GDP are bound. There is no evidence to indicate that these are specific adenylate cyclase activation factors.

Guanylate cyclase-Because of the changes in cyclic nucleotide levels that occur when serum is added to cells, D. Wallach has investigated the effect of serum factors on guanylate cyclase activity. He has found that ether extracts from serum a guanylate cyclase activator. The activator copurifies with fatty acids, and a variety of purified fatty acids at concentrations ranging from 0.1 - 0.5 mM activate the enzyme. It is of particular interest that Mg^{++} ion is the preferred cation for this response.

Insulin binding-In collaboration with P. Thompoulas and J. Roth (NIAMDD) we have measured the ability of Balb 3T3 cells and transformed derivatives to bind ^{125}I-insulin. We find that 5 transformed derivatives all have diminished insulin binding. The fall appears to be due to fewer insulin receptors and not to a change in affinity.

Calcium-A variety of studies have indicated that Ca^{++} may regulate adenylate cyclase and cAMP phophodiesterase activity and modulate the action of cAMP. L. Moore has initiated studies to determine Ca^{++} levels in cells and to determine the activity of the Ca^{++} pump present in microsomal and plasma membranes.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approaches 1, 2 and 5; Objective 4, Approach 2; Objective 6, Approach 3. Various aspects of this work will lead to a better understanding of how cells become cancer cells and how the growth of cancer cells is controlled. It also has therapeutic implications.

Proposed Course: 1. Gather further data on cyclic nucelotide levels in cultured cells and how these levels are controlled. 2. To characterize the prostaglandin receptor. 3. To identify physiologically important molecules that regulate guanylate cyclase activity. 4. To determine the role of calcium in the regulation of cell shape by identifying proteins whose activity is controlled by calcium.

Publications:

Ostlund, R. and Pastan, I.: Fibroblast Tubulin. Biochemistry 14: 4064-4068, 1975.

Miller, Z., Lovelace, E., Gallo, M. and Pastan, I.: Cyclic GMP and Cellular Growth. Science, 190: 1213-1215, 1975.

Nesbitt, J., Anderson, W.B., Miller, Z., Russell, T., Gospodarowocz, D. and Pastan, I.: Guanylate Cyclase and Cyclic GMP Phosphodiesterase Activities and Cyclic GMP Levels in Normal and Transformed Fibroblasts in Culture. J. Biol. Chem., in press.

Thomopoulos, P., Roth, J., Lovelace, E. and Pastan, I.: Insulin Receptors in Normal and Transformed Fibroblasts: Relationship to Growth and Transformation. Cell, in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 8012-02 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Adenylate Cyclase Activity of Transformed and Normal Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wayne B. Anderson, Research Chemist

Ira Pastan, Chief

COOPERATING UNITS (if any)

None

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been devoted to determining how adenylate cyclase activity might be regulated in normal fibroblasts growing in culture. These findings will be of use in trying to elucidate the reasons for altered cyclase activity following viral transformation and during various stages of cell growth. The effects of serum on adenylate cyclase activity is of primary concern since the addition of serum to intact cells decreases intracellular cyclic AMP levels. The addition of serum in vitro selectively inhibits the GTP and hormone-stimulated cyclase activities. Organic solvent extraction of serum resulted in the isolation of a free fatty acid fraction which was very inhibitory toward adenylate cyclase when the free fatty acid to albumin ratio exceeded 5. Unsaturated fatty acids inhibit basal, hormonal, and fluoride activities, while the saturated fatty acids and methyl esters of various chain lengths were without effect. A high molecular-weight factor has been partially purified from serum which demonstrates the selective inhibition of the GTP function; it has little effect on basal or fluoride-stimulated activity. Work is in progress to further purify and characterize this serum factor.

PHS-6040
(12-75)

Serial No. ZO1 CBD 8012-02 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Adenylate Cyclase Activity of Transformed and Normal Fibroblasts

Previous Serial Number: ZO1 CBD 8012-01 LMBGY

Principal Investigator: Wayne B. Anderson

Other Investigators: Ira H. Pastan

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To investigate how adenylate cyclase activity is regulated in normal fibroblasts and how the activity of this enzyme is altered with transformation of fibroblasts by RNA tumor viruses.

Methods Employed: Cell culture and standard biochemical analysis of adenylate cyclase activity.

Major Findings: Previously, we have shown that the properties and activity of fibroblast adenylate cyclase are altered following viral transformation and also during various stages of cell growth. To better understand the mechanisms involved in altering cyclase activity we are attempting to elucidate how this enzyme is regulated in normal fibroblasts growing in culture.

Serum has been found to contain a number of components which alter cyclic nucleotide metabolism. Insulin will decrease cyclase activity if added to intact cells; no effect of insulin on isolated membrane enzyme has been observed. The organic solvent extracted free fatty acid fraction from serum does inhibit the enzyme in vitro. The unsaturated long chain fatty acids markedly inhibit adenylate cyclase activity when the fatty acid to albumin ratio exceeds 5, with the order of effectiveness being arachidonate > linolenate > linoleate >> oleate. The saturated fatty acids and methyl esters of various chain lengths are without effect.

A high molecular weight factor has been partially purified from serum which selectively inhibits the GTP and hormone-stimulated cyclase activity: it has little effect on basal or fluoride-stimulated activity.

Significance for Biomedical Research and the Program of the Institute: Cyclic AMP has an important role in regulating many properties of normal fibroblasts, as well as numerous biological processes in other cell types. Thus, the regulation of adenylate cyclase is of considerable interest. Further, in studying the properties of the normal enzyme we can learn something of the means by which the enzyme has been altered by viral transformation. If it is possible to correct the defective enzyme of transformed cells, this would result in elevated cyclic AMP levels which in turn could revert some of the abnormal properties of cancer cells toward normal.

Proposed Course: We will continue to elucidate the regulatory properties of the fibroblast cyclase system. Particularily, we will further purify and characterize the serum inhibitor and determine its effects on the enzyme from transformed fibroblasts and other cell types. It will also be of interest to determine the effects of the serum factor on other enzymes involved in cyclic nucleotide metabolism. Also, we will determine if it has any effect on the intracellular cyclic AMP levels of intact cells and in this manner might influence many of the properties of normal fibroblasts under cyclic AMP control.

Publications:

Nesbitt, J.A., Anderson, W.B., Miller, Z., Russell, T.R., Gospodarowicz, D., and Pastan, I.: Guanylate Cyclase and Cyclic GMP Phosphodiesterase Activities and Cyclic GMP Levels in Normal and Transformed Fibroblasts in Culture. J. Biol. Chem. 251: in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8010-02 LMBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Role of Cyclic AMP in the Regulation of Morphology, Motility, Malignant Transformation, and Membrane-Mediated Phenomena in Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mark C. Willingham, Senior Investigator

Ira H. Pastan, Chief, LMB
Ken Olden, Senior Staff Fellow

COOPERATING UNITS (if any)

None

LAB/BRANCH

DCBD, NCI, NIH

SECTION

~~Laboratory of Molecular Biology~~

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	.4

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclic AMP produces shape and motility changes in cultured cells, presumably through effects on contractile proteins such as actin and myosin. We have studied the arrangement of these contractile proteins and found that cyclic AMP produces ordered bundle arrays of actin-containing microfilaments and of microtubules. The intracellular arrangement of myosin under these conditions has been studied by immuno-cytochemical and biochemical methods. Myosin is associated with microfilament bundels, the soluble cytoplasm, other cell organelles, and the plasma membrane. We have found that myosin is exposed at both the inside and the outside of the plasma membrane and associated with another new membrane protein. The surface microstructure of these cells has also been studied with cyclic AMP treatment and the changes in agglutination by plant lectins have been linked to changes in surface microvilli caused by cyclic AMP. Since malignant cells in culture often have low cyclic AMP levels, disorganized contractile protein elements, and rapid cell movement, the control of contractile protein functions by cyclic AMP is likely to be highly significant in understanding the nature of malignancy.

Serial No. ZO1 CBD 8010-02 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Role of Cyclic AMP in the Regulation of Morphology, Motility, Malignant Transformation, and Membrane-Mediated Phenomena in Cultured Cells

Previous Serial Number: ZO1 CBD 8010-01 LMBGY

Principal Investigator: Mark C. Willingham

Other Investigators: Ira H. Pastan and Ken Olden

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	1.4
Professional:	1.0
Others:	.4

Project Description:

Objectives: To investigate the mechanisms that control the expression of properties of transformed cells in culture, with particular emphasis on the role of cyclic AMP in modulating these properties.

Methods Employed: Cell culture, specialized light and electron microscopic morphologic and immunohistochemical methods, gel electrophoresis, radiolabelling of cell surface components, and subcellular fractionation procedures.

Major Findings: The past year has been devoted to studying the mechanisms of cell motility, morphology, and lectin agglutination, their changes following malignant transformation, and the effects of cyclic AMP on these properties.

The addition of cyclic AMP to cultured cells produces profound changes in cell shape, motility, and agglutination by plant lectins. The flattened morphology of cells with high cyclic AMP levels is associated with the accumulation of bundles of 70^{o}A microfilaments near the substratum and increased numbers of microtubules. Since microfilament bundles appear to be related to adhesiveness and cell shape changes in response to cyclic AMP, we investigated the proteins associated with them. Actin, the major contractile protein of microfilaments, is likely to be involved in cell movement. We, therefore, investigated the location of the other major contractile

protein, myosin. By immunohistochemistry, we have found that myosin is associated with these microfilament bundles both at the light and electron microscopic levels. However, myosin has been found to be present mainly at the periphery of these bundles, and a great deal of intracellular myosin appears to be associated with other membranes or structures within the cytoplasm or in a freely soluble form. We have observed the association of myosin with the internal surface of the plasma membrane, as well as its presence on the external surface, suggesting that some of the plasma membrane myosin may traverse the entire thickness of the plasma membrane.

To understand further the role of myosin in cell movement, we investigated the presence of myosin in the plasma membrane by biochemical methods. We have found that plasma membrane myosin can be labelled by I^{125}-lactoperoxidase methods from the outside of the cell, separated from other membrane proteins by immunoprecipitation, and identified by gel electrophoresis. However, during this procedure, a new labelled protein associated with myosin was discovered. This new myosin-associated membrane protein, further identified as a glycoprotein with a subunit molecular weight of 210,000, may help anchor myosin into the plasma membrane. During this investigation, a new method was developed that allows direct identification of the protein bands in SDS gels that react with specific antibodies.

We have further investigated the role of cell surface microstructure in transformation and the mediation of the high lectin agglutination of transformed cells. In screening 16 different normal and transformed cultured cell lines by dark field and electron microscopy, we have observed a consistent relationship between transformation and the presence of numerous surface projections such as microvilli. We have also confirmed the lack of redistribution of bound surface lectin (concanavalin A) at both the light and electron microscopic levels during agglutination, and have, thus, reinforced the idea that surface microvilli play the major documented role in the agglutination properties of transformed cells. We have observed the regulation of surface microstructure by cyclic AMP by dark field microscopy and time lapse cinematography, and have concluded that the ability of cyclic AMP to reduce the lectin agglutination of transformed cells is related to its effects on cell surface microstructure.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 6, Approach 3. It appears that a major reason for the many changes in behavior of transformed cells in culture is their inability to maintain normal cyclic AMP levels. By understanding the properties regulated by cyclic AMP, or by raising cyclic AMP levels in these cells, one might control the spread or growth of certain types of cancer.

Proposed Course: We will study the intracellular arrangement of contractile proteins at the ultrastructural level in hopes of better understanding the molecular mechanisms of cell motility and the regulation of these processes by cyclic AMP. We will also study the regulation of other properties of transformed cells, such as surface microstructure, in an

attempt to understand the mechanisms by which cyclic AMP regulated them and how these might relate to the malignant potential of transformed cells.

Publications:

Willingham, M.C. and Pastan, I.: Cyclic AMP Modulated Microvillus Formation and Agglutinability in Transformed and Normal Mouse Fibroblasts. Proc. Nat. Acad. Sci. U.S.A. 72: 1263-1267, 1975.

Willingham, M.C. and Pastan, I.: Cyclic AMP and Cell Morphology in Cultured Fibroblasts. J. Cell Biol. 67: 146-159, 1975.

Willingham, M.C.: Quantitation of Agglutination With an Automatic Particle Counter. H. Bittiger and H.P. Schnebli, eds. Concavalin A as a Tool. John Wiley and Sons, Ltd. (in press) 1976.

Willingham, M.C.: Cyclic AMP and Cultured Cell Behaviour. Int. Rev. of Cytology (in press) 1976.

Olden, K., Willingham, M.C. and Pastan, I. Cell Surface Myosin in Cultured Fibroblasts. Cell (in press) 1976.

Willingham, M.C.: Surface Microstructure of Normal and Transformed Cultured Cells. Exp. Cell Res. (in press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 8210-01 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Role of Plasma Membrane Proteins in the Regulation of Cell Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ira Pastan, Chief, Laboratory of Molecular Biology, DCBD, NCI

Ken Olden, Senior Staff Fellow, Laboratory of Molecular Biology, DCBD, NCI
Jacques Pouyssegur, Visiting Fellow, Laboratory of Molecular Biology, DCBD, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	0

SUMMARY OF WORK (200 words or less - underline keywords)

A mutant fibroblast has been isolated that has diminished adhesiveness to substratum and a round shape. The cell line resembles in appearance cancer cells but does not cause tumors in animals. The round shape is secondary to decreased adhesiveness. The low adhesiveness is due to defective synthesis of many cell surface glycoproteins.

Although most of the myosin of fibroblasts is located in the cytoplasm, 1-2% is located in the plasma membrane. This myosin is probably transmembranal since it can be iodinated by the lactoperoxidase technique.

Serial No. ZO1 CBD 8210-01 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: The Role of Plasma Membrane Proteins in the Regulation of Cell Behavior

Previous Serial Number: None

Principal Investigator: Ira H. Pastan

Other Investigators: Ken Olden and Jacques Pouyssegur

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	2.25
Professional:	2.25
Others:	0

Project Description:

Objectives: To identify the membrane proteins that participate in cell adhesion, cell movement and regulate cell metabolism, and to determine if these are altered in malignant transformation.

Methods: Cell culture; preparation and analysis of membrane proteins and their effects on cell behavior; isolation of mutants with defective membrane proteins.

Major Findings: J. Pouyssegur has isolated mutants of 3T3 cells that have defective adhesion to substratum. These cells are missing a large number of iodinatable surface proteins. The primary defect appears to be in glycoprotein synthesis with a block in glucosamine acetylation. Although these mutant cells have the shape of transformed cells, they have normal growth control and do not form tumors in animals.

K. Olden has used the lactoperoxidase technique to label with ^{125}I cell surface proteins. He finds that one of the proteins iodinated is myosin confirming previous immunological evidence that myosin is a cell surface protein. Preliminary data indicates that myosin may be associated in the membrane with another protein of slightly greater molecular weight.

Significance to Biomedical Research and the Program of the Institute: The invasive properties of tumor cells may be due to altered membrane proteins. Identification and isolation these proteins may suggest new methods of cancer treatment. Objective 3, approach 3.5.

Proposed Course: To isolate further mutants defective in surface properties. To determine the orientation of myosin in the membrane of cultured cells.

Publications:

Pouyssegur, J. and Pastan, I.: Mutants of Balb/c 3T3 Fibroblasts Defective in Adhesiveness. Evidence for an Alteration in Cell Surface Proteins. Proc. Nat. Acad. Sci. USA, 73: 544-548, 1976.

Olden, K., Willingham, M., and Pastan, I.: Cell Surface Myosin in Cultured Fibroblasts. Cell, in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 8011-02 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Structure and Role of a Transformation-Sensitive Cell Surface Glycoprotein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kenneth M. Yamada, Res. Associate

Ira Pastan, Chief

COOPERATING UNITS (if any)
None

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

A major cell surface glycoprotein (CSP) is depleted after viral transformation of fibroblasts in vitro. Isolated CSP will promote cell aggregation, and its addition to a variety of transformed cell types in culture results in partial restoration of fibroblastic morphology, adhesion, and probably contact inhibition of movement. Our objectives will be to examine CSP's role in other aspects of transformation, and to determine the structure of CSP.

Serial No. ZO1 CBD 8011-02 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Structure and Role of a Transformation-Sensitive Cell Surface Glycoprotein

Previous Serial Number: ZO1 CBD 8011-01

Principal Investigator: Kenneth M. Yamada

Other Investigators: Ira H. Pastan

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	1.2
Professional:	1.1
Other:	0.1

Project Description:

Objectives: A major cell surface protein (CSP) is depleted after viral transformation of fibroblasts in vitro. Our objectives are to determine CSP's biochemical structure and its role in cell behavior.

Methods Employed: CSP is isolated from chick embryo fibroblasts using urea, then further purified by salt fractionation and column chromatography at alkaline pH. Agglutinin activity of CSP preparations is determined by hemagglutination. CSP effects on cell-to-substratum adhesion are quantitated by counting the percentage of cells detached after agitation on a gyratory shaker. Contact inhibition of movement is quantitated using the nuclear overlap ratio.

Major Findings: We had previously shown that isolated CSP agglutinated several cell types and was inhibited by proteases and chelating agents, suggesting that CSP is involved in cell-to-cell adhesion of untransformed cells. We have now added CSP back to nine virally transformed cell lines. In general, CSP promotes spreading and elongation to a more fibroblastic morphology, and increases adhesiveness to the substratum. Three cell lines recover parallel alignment of cells accompanied by decreased overlapping of nuclei, even if the CSP is added at concentrations as low as 1 microgram per milliliter of medium. This restoration of a more normal phenotype can be blocked by specific anti-CSP antibodies. CSP did not alter the growth properties of transformed cells.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 5. Neoplastic cells generally display faulty growth control, altered morphology, decreased adhesion, and loss of monolayering and alignment in culture. Our studies show that CSP a major cell surface glycoprotein decreased after transformation of fibroblasts, can partially restore several of these properties when added back to transformed cells, with the apparent exception of growth control. Our results suggest that CSP helps to maintain normal cell morphology, adhesion, and contact inhibition of movement, and that these properties are separable from growth control.

Proposed Course: To examine CSP's effects on other aspects of transformation, including microvillus production and membrane transport and to determine CSP's distribution, structure, biosynthesis, and turnover.

Publications:

Yamada, K.M., Yamada, S.S., and Pastan, I.: The Major Cell Surface Glycoprotein of Chick Embryo Fibroblasts is an Agglutinin. Proc. Nat. Acad. Sci. USA 72: 3158-3162, 1975.

Yamada, K.M., Yamada, S.S., and Pastan, I.: Cell Surface Protein Partially restores Morphology, Adhesiveness, and Contact Inhibition of Movement to Transformed Fibroblasts. Proc. Nat. Acad. Sci. USA (In press), 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8209-01 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Relationship of Carbohydrate and NAD^+ Metabolism to Cell Growth

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

George S. Johnson, Research Chemist

Jose A. Fernandez-Pol, Visiting Fellow

COOPERATING UNITS (if any)

None

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The sugar composition of the culture medium has pronounced effects on cell growth. This object of this proposed work is to understand the biochemical basis for these growth changes. Emphasis will be placed on understanding NAD^+ metabolism and possible effects of sugars on it.

Serial No. ZO1 CBD 8209-01 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Relationship of Carbohydrate and NAD^+ Metabolism to Cell Growth.

Previous Serial Number: None

Principal Investigator: George S. Johnson

Other Investigator: Jose A. Fernandez-Pol

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	2.0
Professional:	2.0
Others:	0.0

Project Description:

Objectives: The sugar composition of the culture medium has pronounced effects on cell growth and metabolism. The object of this project is to delineate the mechanism whereby the sugars or their metabolites influence these properties.

Methods Employed: Standard tissue culture techniques will be used to grow cells. Thin layer chromatography and fluorescent analysis will be used to analyze the metabolic components.

Major Findings: Effects of sugars on cell physiology and growth: Replacement of glucose by other sugars in the culture medium results in rapid and reversible changes in cell appearance and growth. The cells acquire a more flattened and elongated form, and are more adherent to the substratum. Most cell lines grow, but at a reduced rate, when glucose is replaced by other sugars. However one cell line requires glucose for growth. The cells of this cell line can be adapted to grow on sugars other than glucose by simply incubating the cells in medium containing a sugar for a few days. Surprisingly this adapted cell line grows not only on the sugar used during the incubation but on other sugars as well. The molecular basis for this change in phenotype is not clear but the change does not appear to be in the early stages of metabolism of the sugar or in transport.

NAD^+ Metabolism: NAD^+ is an important cellular constituent; however, the extent of its regulatory functions are not clear. It exists in an equilibrium

state with NADH, and the NAD^+NADH ratio influences the activity of oxidation-reduction reactions in the cell. In addition the NAD^+ molecule has a high rate of turnover. The reason of this is not clear but may be related to synthesis of poly ADP-ribose, a nuclear polymer which may be required for cell division.

We have found that the NAD^+NADH ratio is dependent sugar the sugar composition of the culture medium and also cell density. To understand the relevance of these ratio changes we have begun to study the effects of various nicotinic acid derivatives added to the culture medium. Our studies show that these compounds have pronounced effects on cell growth and suggest possible regulatory function for NAD^+ in cell division.

Significance for Cancer Research and the Program of the Institute: By understanding how growth and metabolism are regulated and by understanding differences in this regulation between normal and transformed cells we may learn to control the growth of certain cancer cells.

Proposed Course: Further studies will be done to understand the molecular basis of the effects of sugars on cell growth. Emphasis will be placed on the effects of sugars and nicotinic acid analogues on NAD^+ levels and NAD^+ turnover.

Publications:

Johnson, G.S.: The Importance of Cyclic AMP in Cellular Transformation. B. Weiss, ed.: Cyclic Nucleotides in Disease, University Park Press, Baltimore, pp 35-44, 1975.

Pastan, I., Johnson, G., and Anderson, W.B.: Role of Cyclic Nucleotides in Growth Control. Annual Review of Biochemistry 44: 491-522, 1975.

Johnson, G.S., and Schwartz, J.P.: Effects of Sugars on the Physiology of Cultured Fibroblasts. Experimental Cell Research 97: 281-290, 1976.

Schwartz, J.P. and Johnson, G.S.: Metabolic Effects of Glucose Deprivation and of Various Sugars in Normal and Transformed Fibroblast Cell Lines. Archives Biochem. Biophys., (in press), 1976.

Johnson, G.S.: Cyclic Nucleotides in Normal and Transformed Fibroblasts. W.E. Criss, T. Ono, and J. Sabine, (eds): Modified Cellular and Molecular Control in Neoplasia. Raven Press, N.Y., (in press), 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8000-06 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Regulation of Gene Activity by cAMP and cGMP

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ira Pastan, Chief, Laboratory of Molecular Biology, DCBD, NCI

S. Adhya, Geneticist, Laboratory of Molecular Biology, DCBD, NCI
D. Shlessinger, (Harvard U.)
V. Macchia, U. Naples, Italy

COOPERATING UNITS (if any)
University of Naples, Naples Italy
Harvard University

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	0

SUMMARY OF WORK (200 words or less - underline keywords)

The structure of the cyclic AMP Receptor Protein (CRP) of E. coli is being determined by protein sequencing techniques. The role of cyclic GMP and its regulation is being examined by genetic techniques. Guanylate cyclase activity is regulated by cyclic AMP since the level of guanylate cyclase is increased in cya mutants.

PHS-6040

Serial No. ZOL CBD 8000-06 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Regulation of Gene Activity by cAMP and cGMP

Previous Serial Number: ZO1 CBD 8000-05 LMBGY

Principal Investigator: Ira Pastan

Other Investigators: S. Adhya, D. Shlessinger, and V. Macchia

Cooperating Units: Harvard University
University of Naples, Naples Italy

Man Years: July 1, 1975 through June 30, 1976

Total:	.75
Professional:	.75
Others:	0

Project Description:

Objectives: Cyclic AMP is required for the synthesis of a variety of enzymes in E. coli, most of which are inducible. Cyclic AMP acts at a transcriptional level. Our objective is to determine how cyclic AMP, cyclic AMP receptor protein (CRP) and other factors control gene activity.

Methods Employed: Studies on mRNA and enzyme synthesis have been carried out in intact cells and in cell-free extracts. Mutants which are defective in cyclic AMP metabolism have been isolated and characterized. Proteins controlling gene transcription are isolated and their mechanism of action studied.

Major Findings: Studies on the sequence of CRP are continuing. To supplement previous methods trypsin digests have been prepared, the peptides separated, isolated and are now being analyzed. V. Macchia has purified guanylate cylcase from E. coli. Apparently guanylate cyclase activity is regulated by cyclic AMP since enzyme activity is increased 3-4 fold in cya and crp mutants.

Significance to Biomedical Research and the Program of the Institute: National Cancer Plan Objective 2, Approach 3. As we understand in detail how the expression of bacterial genes is controlled we will be able to understand and then control the expression of the genes of animal cells and modify the behavior of cancer cells.

Proposed Course: To complete the structure of CRP and examine how it interacts with promoters. To elucidate the biological role of cyclic GMP.

Publications:

Nakanishi, S., Adhya, S., Gottesman, M., and Pastan, I.: Selective Effects of MgCl2 and Temperature on the Initiation of Transcription at Lac, Gal and λ Promoters. J. Biol. Chem. 250: 8202-8208, 1975.

Macchia, V., Varrone, S., Weissbach, H., Miller, D. and Pastan, I.: Guanylate Cyclase in Escherichia Coli: Purification and Properties. J. Biol. Chem. 250: 6214-6217, 1975.

Pastan, I., and Adhya, S.: Cyclic AMP in E. coli. Bacteriol. Rev., in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8006-05 LMBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Control of Expression of the Galactose Operon in E. coli K12, Bacteriophage λ and P22

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Sankar L. Adhya, Geneticist
Max E. Gottesman, Head, Biochem. Genetics Sect.,
Donald L. Court, Staff Fellow

Asis Das, Visiting Fellow
Oscar Reyes, Visiting Fellow
Sandra Hilliker, Guest Worker

COOPERATING UNITS (if any)

None

LAB/BRANCH

DCBD, NCI, NIH

SECTION

Laboratory of Molecular Biology

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

We have previously suggested that the gal operon of E. coli has two promoters one promoter is subject to positive control by cyclic AMP and cyclic AMP receptor protein and the other promoter to negative control by the cyclic AMP nucleotide and its receptor protein. The structure of these promoters is being analyzed. We have shown that the operon also carries signals for transcription termination by the termination factor rho not only at the end of the operon but also within it. We have proposed that polarity of gene expression of an operon is because of premature transcription termination within an operon. This is supported by the isolation and characterization of various types of E. coli mutants defective in transcription termination factor rho.

The control of gene expression in temperate bacteriophages λ and P22 is also based on overcoming transcription termination at specific signals. A similar regulatory gene has been identified in λ and P22 that prevents transcription termination. We are analyzing the mechanisms of action of these regulatory genes. We are also studying the effect of λ development on host cell physiology.

Serial No. ZO1 CBD 8006-05 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Control of Expression of the Galactose Operon in E. coli K12 Bacteriophages λ and P22.

Previous Serial Number: ZO1 CBD 8006-04

Principal Investigators: Sankar L. Adhya, Max E. Gottesman and Donald L. Court

Other Investigators: Asis Das, Oscar Reyes and Sandra Hilliker

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	6.0
Professional:	6.0
Others:	0.0

Project Description:

Objectives: The gal operon of E. coli is normally subjected to both a positive control and a negative control by cyclic AMP and its receptor protein. The operon may also be subjected to a different type of control whereby expression of distal genes is regulated by the amount of transcriptional read-through at various termination signals located within the operon. Our object is to analyze the control signals--initiation and termination--of the gal operon and identify the regulatory molecules that interact with these signals. We are also studying the expression of genes in temperate bacteriophages λ and P22 and their effect on the transformation of host cell physiology and gene expression.

Methods Employed: Standard microbial genetic and biochemical techniques.

Major Findings: Based on our previous findings, we have proposed a general mechanism of transcription termination. The model states that all (both host and phage) transcription termination is effected by protein factor rho. Rho acts by attaching to nascent messenger RNA, exposed by dissociation of ribosomes at polypeptide chain terminating codons, and subsequently directs RNA polymerase to stop transcription. We have isolated E. coli mutants defective in rho. Mutations in rho cause read through transcription where termination would normally occur.

Transcription termination by rho can be overcome by a gene product of phage λ and P22. The biochemical mechanism of action of the phage gene

products which cause this are now being investigated. Host mutations have been isolated which prevents antitermination by phage.

A λ gene has also been identified the product of which not only increases the stability of λ messenger RNA, but also increases ppGpp levels in the cell, decreases ribonucleoside uptake into the cellular ribonucleoside triphosphate pool, and decreases cellular retention or synthesis of cyclic AMP.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Object 3, Approach 1. In cancer cells, the expression of some genes are permanently turned on, i.e. expressed constitutively. Our studies are aimed to understand the molecular basis of how genes are turned on and off. We are using gal as a model system. This understanding might help to prevent conversions of cells to those capable of forming cancers.

Proposed Course: 1. To determine the complete structure of the gal operon. 2. To identify all the regulatory molecules involved in turning on and off the various genes of the gal operon, phage λ and P22. 3. To understand the biochemistry of the initiation and termination signals of gene expression. 4. To understand the transformations caused by phage growth on its host.

Publications:

Nakanishi, S., Adhya, S., Gottesman, M., Pastan, I.: Selective Effects of $MgCl_2$ transcription and temperature on the initiation of Transcription at Lac, Gal and λ promoters. J. Biol. Chem, 250, 8202-8208, 1975.

Adhya, S., Court, D., de Crombrugghe, B. and Gottesman, M.: Changes Transcription of Host Genes After Bacteriophage Lambda Induction. Fed. of European Biochem. Societies, 69-73, 1975.

Court, D., Green, L., and Echols, H.: Positive and Negative Regulation by the cII and cIII Gene Products of Bacteriophage λ. Virology 63, 484-491, 1975.

Adhya, S., Gottesman, M., de Crombrugghe, B. and Court, D.: Transcription termination regulates gene expression in RNA Polymerase. M. Chamberlin & R. Losick, ed. Cold Spring Harbor Laboratory (in press), 1976.

Adhya, S., Court, D., Das, A.: Isolation and Characterization of Conditional Lethal Mutants of E. coli Defective in Transcription Termination factor rho. Proc. Nat. Acad. Sci.(in press), 1976.

Reyes, O., Gottesman, M. and Adhya, S.: Suppression of Polarity of Insertion mutations in the gal operon and N mutations in phage λ. J. Bact. (in press), 1976.

Pastan, I. and Adhya, S.: Cyclic AMP in E. coli. Bacteriol, Rev., (in press), 1976.

Echols, H., Court, D. and Green, L.: On the Nature of cis-Acting Regulatory Proteins and Genetic Organization in Bacteriphage: The Example of Gene Q of Bacteriophage λ. Genetics, (in press), 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8008-04 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Initiation and Termination of Transcription of Bacterial Operons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Benoit de Crombrugghe, Senior Investigator; Richard Musso, Staff Fellow; Bruce Howard, Research Associate; Roberto DiLauro, Visiting Fellow; and Martin Rosenberg, Staff Fellow, Laboratory of Molecular Biology, DCBD, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	4.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

We study the transcription of the E. coli galactose operon as a model system for gene regulation. This operon is subject to both negative regulation by a specific repressor protein and to positive control by cyclic AMP and the cyclic AMP receptor protein (CRP). We attempt to define the precise protein-DNA interactions which control the regulation of this operon. The nucleotide sequence of the regulatory region of the galactose operon has been determined. There are two closely spaced startpoints for transcription in the galactose operon and presumably two promoters; one controlled by CRP and cyclic AMP, the other independent of those factors. We are presently defining which nucleotides in the sequence we have determined interact respectively with the gal repressor, RNA polymerase and CRP.

Serial No. Z01 CBD 8008-04 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Studies on Initiation and Termination of Transcription of Bacterial Operons

Previous Serial No.: 8008-03

Principal Investigators: Benoit de Crombrugghe, Senior Staff Investigator; Richard Musso, Staff Fellow; Bruce Howard, Research Associate; Roberto Di Lauro, Visiting Fellow; Martin Rosenberg, Staff Fellow

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	4.1
Professional:	4.1
Other:	0.0

Project Description:

Objective: To understand the precise biochemical mechanisms which control initiation and termination of transcription.

Methods Employed: Measurement of RNA and protein synthesis in vivo and in cell-free systems; isolation of factors active in regulation of initiation and termination of transcription; purification of specific DNA fragments; purification of specific RNA species; nucleotide sequence analysis of RNA and DNA; use of probes for the study of protein-nucleic acid interaction.

Major Findings: (a) The galactose (gal) operon of E. coli is regulated by an operon specific repressor and by cyclic AMP and the cyclic AMP receptor protein. In addition, other cellular components probably regulate gal operon expression. We have sought to determine the nucleotide sequence of the control region of the gal operon and to define the functions of the segments of this region which bind gal repressor, the cyclic AMP-CRP complex and RNA polymerase.

Using a series of restriction endonucleases we first established a restriction map of the gal regulatory region. We had earlier determined the nucleotide sequence of the first 77 bases at the 5' end of gal mRNA. This RNA was synthesized in vitro in a kinetic reaction in the presence of cyclic AMP and CRP.

Using both RNA and DNA sequencing methods we have established the nucleotide sequence of a segment of 92 bases in the DNA preceding the sequence determined earlier. This region includes the genetic information needed for cyclic AMP-CRP stimulation of gal transcription.

The DNA sequence (presented as the non-template strand) is as follows:

```
                -80        -70        -60        -50        -40        -30
5' GCGCAGAAAACGGCTAAATTCTTGTGTAAAAGATTCCACTAATTTATTCCATGTCACACTTT
       -20        -10         0+1        +10        +20        +30
TCGCATCTTTGTTATGCTATGGTTATTTCATACCATAAGCCTAATGGAGCGAATTATGAGTTCT
```

Cyclic AMP-CRP stimulated transcription starts at position +1. Restriction endonucleases Hinf and Hha cleave respectively between residues -60 and residue -59, and between residue -89 and residue -88. Cleavage by Hinf abolishes cyclic AMP-CRP dependent transcription of gal but not cyclic AMP-CRP independent transcription. Formation of a cyclic AMP-CRP dependent preinitiation complex prevents cleavage by Hinf; formation of a cyclic AMP-CRP independent preinitiation complex does not prevent cleavage by Hinf. Thus, the region around -60 is important for cyclic AMP-CRP stimulation of transcription. Furthermore, comparison of this segment with the presumed CRP-cyclic AMP binding site in the lac operon shows important similarities, suggesting that the region between residue -75 and residue -48 may be important for CRP recognition in gal.

We have analyzed four gal operator mutants: two of them are resistant to cleavage by Hinf endonuclease. The base changes in two others were determined. In one O^c mutant residue -63 and residue -64 are changed respectively to T and G. In the other O^c mutant residue -66 is changed to A. Thus, the gal operator is located in a segment of the DNA which includes at least residue -66 to residue -59. This suggests that the repressor binding site may overlap with the CRP binding site.

We have also obtained evidence that CRP-cyclic AMP independent transcription of gal starts at residue -5, thus at a different place than the CRP-cyclic AMP dependent transcription, which starts at +1. Transcription starting at +1 is totally dependent on CRP-cyclic AMP. Furthermore CRP and cyclic AMP abolish while glycerol stimulates transcription starts at -5. Cleavage at the Hinf site does not prevent CRP-cyclic AMP independent starts at -5, but abolishes cyclic AMP-CRP dependents starts at +1. A heptanucleotide, similar to those present in other promoters preceding the startpoint of transcription, is found at residues -12 to -6 preceding the CRP-cyclic AMP dependent transcription startpoint by 5 residues. An analogous heptanucleotide is found at residue -17 to -11, preceding the CRP-cyclic AMP independent transcription startpoint by the same distance. These experiments suggest the existence of two interelated promoters in the gal operon; they also suggest a new working hypothesis which could clarify several hitherto unexplained features of the in vivo regulation of gal.

Present studies include: (1) determination of base changes in several operator and promoter mutants; (2) definition of the precise bases in the DNA which interact with repressor, CRP and RNA polymerase; (3) analysis of the mode of action of the gal repressor and of cyclic AMP-CRP on both cyclic AMP-CRP dependent and independent transcription; (4) comparison of sequences of in vivo made RNA's with in vitro synthesized RNA's.

(b) Termination of transcription occurs at defined DNA sequences located at the 3' end of operons (or cistrons). With E. coli or bacteriophage DNA, the protein factor rho is required for proper termination by RNA polymerases at many of these sites. An RNA dependent nucleotide phosphohydrolase (ATPase) was found to be associated with rho (Lowery et al., 1974). Our experiments indicate that the hydrolysis of the $\beta\gamma$ phosphate bond of a ribonucleoside triphosphate is required for rho mediated termination of transcription. Present studies aim at (1) defining the role of the ATPase activity in the transcriptional termination process. (2) determining the nucleotide sequence around rho dependent termination sites in λDNA and gal DNA.

Significance for Cancer Research and the Program of the Institute: We study the regulation of a particular operon of E. coli as a model for control of gene expression. We determine the nucleotide sequence of the regulatory region of the gal operon, both of the wild type DNA and of gal mutants affected in several functions of the control region. We also define the segments of this DNA which interact with the regulatory proteins controlling gal expression. These studies will help us understand the role of the DNA-protein interaction which govern the regulation of this operon. We anticipate that similar protein-DNA interactions control gene expression in normal and transformed animal cells.

Proposed Course: (1) Study the regulation of the galactose operon of E. coli by determining the nucleotide sequences of the regulatory segments of both wild type and mutant gal DNA, and by analyzing the interactions between the regulatory proteins and their DNA binding sites. (2) Search for additional factors which are active in the regulation of this operon, and determination of their mode of action. (3) Study the mechanisms which regulate termination of transcription in E. coli. (4) Purify the mRNA and later the gene which specifies an important cell surface protein in chick cells; this protein is present in normal cells but greatly reduced in transformed cells probably as a result of decreased synthesis; we want to study the regulation of the synthesis of this protein using analogous methods as those we utilized for the study of the gal operon in E. coli.

Publications:

De Crombrugghe, B.: DNA Dependent Cell-Free Protein Synthesis. Protein Biosynthesis, Academic Press, 1975.

Rosenberg, M., Weissman, S. and de Crombrugghe, B.: Termination of Transcription in Bacteriophage Lambda. J. Biol. Chem. 250: 4755-4764, 1975.

Rosenberg, M., de Crombrugghe, B. and Musso, R.: Determination of Nucleotide Sequences Beyond the Sites of Transcriptional Termination. PNAS (1976) in press.

Adhya, S., Gottesman, M., de Crombrugghe, B. and Court, D.: Transcription Termination Regulates Gene Expression. RNA Polymerase, Cold Spring Harbor, N.Y., R. Losick and M. Chamberlin (Eds.), in press.

Howard, B. and de Crombrugghe, B.: ATPase Activity Required for Termination of Transcription by the E. coli Protein Factor rho. J. Biol. Chem. (1976) in press.

Di Lauro, Roberto, Musso, Richard, Rosenberg, Martin and de Crombrugghe, Benoit: DNA Sequence of the Regulatory Region of the Galactose Operon. Federation Proceedings (1976) in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8013-02 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
An Examination of E. coli Polymerase Mediated Initiation and Termination of Transcription and Post-transcriptional Processing of Certain RNA Species.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Martin Rosenberg, Staff Fellow

Benoit de Crombrugghe, Senior Investigator

COOPERATING UNITS (if any)

None

LAB/BRANCH
DCBD, NCI, NIH

SECTION
Laboratory of Molecular Biology

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

We have been investigating nucleic acid structure involved in the control of transcriptional initiation, termination, and post-transcriptional processing and modification of various RNA transcription products. Nucleic acid sequences have been determined in the 3'-terminal regions of a number of bacterial RNA polymerase mediated transcription products synthesized from λ and λ gal transducing phages. We have also examined nucleic acid structure in the "terminator" regions of the DNA template beyond the 3'-ends of several RNA transcription products. We are presently defining the structure surrounding an RNA polymerase stop-signal in bacteriophage λ which is totally dependent on the protein termination factor, rho, for its function. Nucleic acid structure in the regulatory region of the galactose operon of E. coli has also been examined.

In addition, the nucleotide sequence of a region of the bacteriophage T7 genome which lies between two adjacent "early" messenger RNAs has been completed. This intercistronic region contains all of the structural information necessary for specifying the processing event (i.e. cleavage by the host encoded endonuclease RNase III) which matures this polycistronic precursor RNA into individual monocistronic mRNAs.

PHS-6040
(10-75)

Serial No. ZO1 CBD 8013-02 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: An Examination of E. coli Polymerase Mediated Initiation and Termination of Transcription and Post-transcriptional Processing of Certain RNA Species.

Previous Serial Number: ZO1 CBD 8013-01 LMBGY

Principal Investigator: Martin Rosenberg

Other Investigators: Benoit de Crombrugghe

Cooperating Units: None

Man Years: July 1, 1975 through June 30, 1976

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description:

Objectives: 1) To determine the nucleic acid structure involved in the control of transcriptional termination and post-transcriptional modification of RNA and to understand the functional relationship of this structure to the regulation of these processes.

2) To determine the nucleic acid structure involved in the regulation of gene expression of the galactose operon of E. coli.

3) To develop new techniques which can be applied to both these studies and to the general examination of nucleic acid structure in other biological systems.

Methods Employed: Purification of specific RNA transcripts synthesized either in vivo or in vitro; nucleic acid sequence analysis of both RNA and DNA; primed extension of RNA transcription products with deoxyribonucleotides; isolation of protein factors involved either in the processes of transcriptional termination or post-transcriptional modification of RNA.

Major Findings: 1) The nucleic acid primary structure at the termini of the bacteriophage T7 "early" mRNAs synthesized both in vivo and in vitro have been examined. The sequence information obtained supports

a model for transcription of the "early" region in which a high molecular weight precurser RNA molecule is post-transcriptionally processed to yield monocistronic messengers. The processing agent is a host specified endonuclease, Ribonuclease III, which is also involved in the post-transcriptional processing of a ribosomal RNA precursor in E. coli. Thus, it was shown that the T7 "early" RNAs are direct products of RNase III cleavage of a large polycistronic precursor molecule.

In addition, we demonstrated that the 3'-termini of the processed RNAs contained sequence heterogeneity arising from the addition of several (up to 5) adenylate residues to the 3'-end. These A additions appear to be a non-template specified, post-transcriptional, post-processing, modification of the RNA.

In an effort to further elucidate the mode of action of RNase III in the processing event, as well as examine the entire structure of an intercistronic region of the phage, we have now isolated and sequenced an RNA fragment which traverses the region between two adjacent "early" T7 genes (thereby spanning an RNase III cleavage site).

Sequence analysis of this fragment indicates that the processing enzyme produces a single endonucleolytic cleavage in this region giving rise directly to the GO.3 and GO.7 T7 mRNAs. In addition, the 3'-terminal adenylic acid residues observed previously on the in vivo T7 early RNA species were not found in these fragments and, therefore, must represent a post-transcriptional, post-processing modification of the RNA.

The secondary structure of this region is also quite characteristic. Two relatively stable base-paired stretches of RNA adjoin the cleavage site in a somewhat symetrical arrangement. These double-stranded regions appear to be required for recognition of the cleavage site by RNase III. The site of scission however is located in a small region of RNA set between the double-stranded regions.

2) The nucleic acid structure of the 3'-terminal regions of a number of RNA transcripts synthesized in vitro from λ and λ gal transducing phages are being examined. These RNAs represent both independently terminated transcription products as well as transcripts requiring the protein factor, Rho, for their termination. Sequence analysis at the 3'-ends of some of these products has indicated:

a) that all of the transcripts examined possess similar degrees of 3'-terminal sequence hetereogeneity which consisted predominately of the addition of 1 to 5 adenylate residues to the 3'-terminus of the transcript and b) that rho factor enhanced termination results in a definite structural change in the nucleotide sequence with which an RNA molecule can terminate.

In addition, we have developed a new methodology for examining the nucleic acid structure in the untranscribed portion of the DNA immediately adjacent to the 3'-terminus of an RNA transcript. This technique has been applied to the determination of sequence information throughout the entire termination region

of these transcription products. Little structional homology is apparent in the template DNA beyond the 3'-ends of these transcripts. The lack of homology suggests that this region might not be important to the termination process. Thus, the majority of the encoded termination signal is transcribed into RNA.

3) The galactose operon of E. coli is subject to both negative control by gal repressor and positive control by cAMP and its receptor protein (CRP). We have sought to define the nucleotide sequence of the promoter-operator sites responsible for gal regulation. Analysis of transcripts of restriction fragments combined with direct DNA sequencing enabled us to derive the sequence for the gal regulatory region. Cleavage by restriction endonuclease Hin f abolishes cAMP-CRP dependent but not CRP independent transcription. Formation of a cAMP-CRP dependent preinitiation complex prevents cleavage by Hin f. Thus the region upstream of the Hin f site is important for cAMP-CRP stimulation of transcription. This region contains sequence similarities with the CRP recognition site of lac. One gal operator constitutive mutant is resistant to Hin f cleavage suggesting the operator may include at least part of the Hin f site. Further analyses are required to confirm this possibility.

Significance for Cancer Research and the Program of the Institutes: National Cancer Plan Objective 3, Approach 1. The regulation of gene expression in both prokaryotic and eukaryotic systems involves numerous and complex structural and functional interactions between nucleic acids and proteins. It is highly probable (and experimental evidence increasingly supports the contention) that many of the nucleic acid fine-structure interactions which occur on the prokaryotic level have basic similarities to analogous type interactions known to occur in higher cells. Our studies involve the determination of the nucleic acid structure involved in the control of the initiation and termination of transcription by RNA polymerase and the post-transcriptional processing of certain RNA species by specific endonucleases (e.g. RNase III) in both bacterial and related viral systems. The elucidation of these transcriptional processes and their regulatory elements will be a basis for understanding similar processes occurring in eukaryotic cells (both normal and transformed) and virus (oncogenic and other).

Proposed Course: To examine further the mechanisms by which RNA transcripts are initiated, terminated and subsequently processed during the bacterial and viral life cycle.

Publications:

Rosenberg, M., Kramer, R.A., and Steitz, J.A.: The specificity of RNase III cleavage of bacteriophage T7 early messenger RNAs. Brookhaven Symp. in Biol. 26: 277-285, 1975.

Rosenberg, M., de Crombrugghe, B., and Weissman, S.: Termination of transcription in bacteriophage lambda: Heterogeneous, 3'-terminal oligadenylate additions and the effect of rho factor. J. Biol. Chem., 4755-4764, 1975.

Sogin, M.L., Pace, N.R., Rosenberg, M., and Weissman, S.M.: The Nucleotide Sequence of a 5S Ribosomal RNA Precursor from Bacillus subtilis. J. Biol. Chem., 1976, in press.

Rosenberg, M., de Crombrugghe, B., and Musso, R.: Determination of Nucleotide Sequence Beyond the Sites of Transcriptional Termination. Proc. Nat. Acad. Sci., March, 1976 in press.

Kramer, R., and Rosenberg, M.: The Isolation and Characterization of Bacteriophage T7 mRNA Fragments Containing an RNase III Cleavage Site. Nucleic Acid Research, 1976 in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8208-01 LMBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Molecular Genetics of Immunoglobulin Biosynthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marc Shulman, Staff Fellow

COOPERATING UNITS (if any)

None

LAB/BRANCH

DCBD, NCI, NIH

SECTION

Laboratory of Molecular Biology

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques are being developed to elucidate the primary structure of DNA encoding immunoglobulin genes.

Serial No. ZO1 CBD 8208-01 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Molecular Genetics of Immunoglobulin Biosynthesis

Previous Serial Number: None

Principal Investigator: Marc Shulman

Other Investigator: None

Cooperating Unit: None

Man Years: July 1, 1975 through June 30, 1976

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To develop techniques to elucidate primary structure of DNA encoding immunoglobulins in germ line and plasma cells.

Methods Employed: If translocation, mutation, or recombination of immunoglobulin genes occurs as the antibody capacity develops, then the nucleotide sequence of immunoglobulin mRNA will differ from that of the immunoglobulin germ line genes. Using mRNA and DNA from wild type and mutant phage T7, I am constructing molecules analogous in structure to those expected for hybrids of immunoglobulin mRNA and germ line DNA if somatic variation underlies immunoglobulin diversity. I am digesting these molecules with various nucleases to ascertain if the different structures can be distinguished by different sensitivity to these nucleases.

Significance for Cancer Research and the Program of the Institutes: By recognizing the novel antigenic properties of cancer cells, the immune system might serve to block the development of such cancers. Understanding how the immune system develops might therefore indicate how the organism normally protects itself against cancer and thus indicate deficiencies in those individuals susceptible to cancer.

Publications:

Fiandt, M., Gottesman, M.E., Shulman, J.J., Szybalski, E.H., Szybalski, W. and Weisberg, R.A.: Physical Mapping of Coliphage λ att^{2}. Virology, in press.

Shulman, M., Mizuuchi, K. and Gottesman, M.M.: New att Mutants of Phage λ. Virology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8401-13 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Endocytosis in the Thyroid Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Seymour H. Wollman, Head, Cell Organization Section, Laboratory of Molecular Biology, DCBD, NCI

Joseph D. Zeligs, Research Associate, Cell Organization Section, Laboratory of Molecular Biology, DCBD, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Cell Organization Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The typical thyroid epithelial cell can take in colloid from the follicular lumen by macropinocytosis. It can also phagocytose red blood cells. We propose to study the mechanism of these processes by electron microscopy, histochemistry and related techniques.

PHS-6040
(12-75)

Serial No. ZO1 CBD 8401-15 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Endocytosis in the Thyroid Gland

Previous Serial Number: ZO1 CBD 8401-14 LMBGY

Principal Investigator: Seymour H. Wollman

Other Investigators: Joseph D. Zeligs

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objectives: To determine mechanism of thyroid hormone release, and more generally, endocytotic properties of typical thyroid epithelium.

Methods: We have made electron microscope observations on cytoplasmic projections from the apical end of the epithelial cell into the lumen of the follicle in the hyperplastic thyroid gland.

Major Findings: Blebs similar to zeiotic blebs occurring in mitotic cells in tissue culture were observed in hyperplastic thyroid epithelial cells in vivo. They were relatively dark, rounded cytoplasmic projections into the lumen from the apical surface. They contained an array of microfilaments at the periphery, a considerable but variable concentration of free ribosomes and an occasional crystalloid. There were other dark cytoplasmic processes extending into the follicular lumen that resembled a pseudopod in cytoplasmic content. There was some suggestion of possible conversion of pseudopod to bleb. There was also evidence of attempted phagocytoses of these structures by cells from the opposite side of the follicular lumen, such cells being only a few micrometers away in the hyperplastic gland.

Induction of pseudopods by contact: In the very hyperplastic thyroid gland colloid is largely depleted from the follicle. The distance between cells on opposite sides of the follicle may be as small as 1μm. In such follicles there is a relatively high incidence of pseudopods in pairs in close contact, one pseudopod from each of 2 cells on opposite sides of the lumen. Occasionally there was just a single pseudopod in contact with the

the apical surface of a cell from the opposite side of the lumen. At points of contact there was indication of an early stage of pseudopod formation. These observations suggest that pseudopods can be induced in the apical surface of a cell by contact with another pseudopod.

Significance for Cancer Research and the Program of the Institute: This project fits most closely under Objective 3, Approach 5, having to do with properties of the cell surface.

Proposed Course: Further studies of the induction and nature of pseudopods will be undertaken.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 905-24 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Thyroid Hormone Synthesis and Storage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Seymour H. Wollman, Head, Cell Organization Section, Laboratory of Molecular Biology, DCBD, NCI

Ragnar Ekholm, Visiting Scientist; Andre Haeberli, Visiting Fellow, Cell Organization Section, Laboratory of Molecular Biology, DCBD, NCI, NIH

Lois Tice, Lab. Exp. Path., NIAMDD, NIH, Bethesda, MD.

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Cell Organization Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

This project has as its objectives to learn about: (1) The mechanism by which the thyroid gland maintains a concentration of iodide elevated above that of the blood, and (2) The mechanism by which the thyroid gland forms and accumulates protein-bound iodine.

The work is largely based upon in vivo studies and makes use of autoradiography, cytochemistry at ultrastructural level, kinetic studies, and inhibitors of steps in the synthesis of thyroid hormones.

PHS-6040
(10-75)

Serial No. ZO1 CBD 905-24 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Thyroid Hormone Synthesis and Storage

Previous Serial Number: ZO1 CBD 905-23 LMBGY

Principal Investigator: Seymour H. Wollman

Man Years: July 1, 1975 through June 30, 1976

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To learn about the mechanism of synthesis and storage of thyroid hormones.

Major Findings: None

Proposed Course: To be resumed in the future.

Significance for Cancer Research and the Program of the Institute: Some of the work reported here may best be put into Objective 3, Approach 5 because they give information on properties of cell surface and cell membranes.

Publications:

Ekholm, R. and Wollman, S.H.: Site of Iodination in the Rat Thyroid Gland Deduced from Electron Microscopic Autoradiographs. Endocrinology 97: 1432-1444, 1975.

Tice, L.W. and Wollman, S.H.: Changes in Tight Junctions of the Thyroid Epithelium with Changes in Thyroid Activity. J. Cell Biol. 66: 657-663, 1975.

Tice, L.W. and Wollman, S.H.: Gap Junctions in Thyroid Epithelium of the Rat. Endocrinology 98: 800-801, 1976.

Bernstein, L.H. and Wollman, S.H.: Association of Mitochondria with Desmosomes in the Rat Thyroid Gland. J. Ultrastructure Research 53: 87-92, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 906-24 LMBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Production and Properties of Transplantable Thyroid Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Seymour H. Wollman, Head, Cell Organization Section, Lab. Mol. Biol., DCBD, NCI
Joseph D. Zeligs, Research Associate, Cell Organization Section, Lab. Mol. Biol. DCBD, NCI
S.R. Hilfer, Dept. of Biol., Temple Univ., Philadelphia, Pa.
J.P. Herveg, Guest Worker, Cell Organization Section, LMB, DCBD, NCI, NIH
F.S. Ambesi-Impiombato, Visiting Associate, Consultant, Cell Organization Section, LMB, DCBD, NCI, NIH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Cell Organization Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

To study the thyroid growth and the production and properties of transplantable thyroid tumors in the rat: changes occurring during growth, hyperplasia and involution, growth and properties of thyroid cells and thyroid tumor cells in culture.

We are also studying a variety of types of cells just recently recognized to be present in the normal thyroid gland and which may be the cells of origin of some of the different histologic types of thyroid tumors which have been observed in the past.

PHS-6040
(12-75)

Serial No. Z01 CBD 906-24 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Production and Properties of Transplantable Thyroid Tumors

Previous Serial No.: Z01 CBD 906-23 LMBGY

Principal Investigator: Seymour H. Wollman

Other Investigators: J.D. Zeligs, S.R. Hilfer, J.P. Herveg, F.S. Ambesi-Impiombato, and H. Coon

Cooperating Units: Department of Biology, Temple University
Philadelphia, Pennsylvania, and
Laboratory of Cell Biology, DCBD, NCI

Man Years: July 1, 1975 through June 30, 1976

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description:

Objectives: To study thyroid growth and the production and properties of transplantable thyroid tumors in the rat: changes occurring in the thyroid during growth, hyperplasia and involution. We are also studying a variety of cells recently recognized to be present in the normal thyroid gland and which may be the cells of origin of some of the different histologic types of thyroid tumors observed in the past. We are also trying to grow normal and neoplastis thyroid epithelium in vitro.

Methods Employed: Origin of cell types in thyroid: Ventral and ultimobranchial outpocketings of the embryonic pharynx were removed prior to their fusion to form the developing thyroid gland. They were implanted separately in the kidney capsule of the adult and allowed to differentiate. Later they were removed and examined by electron microscopy. Hyperplasia: Hyperplasia was produced in rat thyroid gland by feeding of the goitrogen, thiouracil. Various details of the nature of the growth process were examined by electron microscopy. Details included the nature of the miotic process in epithelial and endothelial cells. Tissue culture: Cells were grown in vitro in the presence or in the absence of a fibroblast feeder layer. Stereology of the hyperplastic thyroid: Quantitative changes in the number and volume of epithelial cells, volume of blood vessels and follicular

lumens were determined on histologic sections during development of hyperplasia. Origin of cell types (with Hilfer): A second type of ultimobranchial follicle was recognized in transplants of ultimobranchial outpocketings of embryo rats into the kidney capsule of the adult. This follicle has a thick wall made up primarily of calcitonin cells but the latter are separated from the lumen by a flat cell with cytoplasm containing an abundance of filaments. Hyperplasia (with Zeligs): Microhemorrhage occurs during early stages of hyperplasia, after feeding a goitrogen for between 3 and 10 days. The red blood cells (RBCs) seem to emerge from the capillaries one by one squeezing through a narrow opening in the capillary wall. The RBCs lodge between the follicular epithelial cells and some enter the follicular lumen. The leakage of RBCs from the capillary may be related to old observations of the microhemmorhage that occurs in growing capillaries.

(With Herveg) During the development of hyperplasia in the thyroids of rats fed a goitrogen for 3 weeks the major change is an increase in the volume occupied by capillaries (a 40-fold increase while the gland volume increases by 6X). The volume occupied by epithelial cells increases 4X but only half is due to a doubling of cell molecules, the remainder to an increase in volume of each epithelial cell. The fraction of the gland occupied by follicular lumens decreases by a factor of 4.

Mitosis (with Zeligs): We have examined changes occurring in cytoplasmic organelles during mitosis in the thyroid epithelial cell. The extensive rough ER breaks up into small rough spherical vesicles in prephase and reforms in telophase. The Golgi apparatus becomes a compact cloud of 500 Å vesicles which is partitioned into the daughter cells and reforms the typical Golgi apparatus at telophase. On the other hand, the lysosomes do not appear to fragment, but they migrate to the periphery of a central plane perpendicular to the spindle and are also partitioned into the daughter cells at telophase. These changes seem to be different mechanisms for the partition of cytoplasmic organelles into daughter cells at telophase.

Culture of normal and neoplastic thyroid epithelial cells in vitro (with Ambesi and Coon). Typical thyroid epithelial cells can be grown in a modified F_{12} medium with 5% calf or horse serum in the presence of a feeder layer of irradiated fibroblasts. In such a system they form 3-dimensional structures with lumens and secrete thyroglobulin. The epithelial cells can also be grown on cover slips physically separated from but in the culture flask with fibroblasts. They can also be grown in the absence of fibroblasts, but in a medium conditioned by the prior growth in it of fibroblasts in the presence of dibutyryl cyclic AMP. Cells of certain restricted lines of thyroid tumors can be grown in the modified F_{12} medium with 5% calf serum but others do not. Almost all the cells are killed by freezing under conditions in which the common lines of cells survive.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 1. The properties of cells and of the tumors derived from them frequently depend upon the embryo-logic origin of the cells. This project will allow us to understand better the

properties of certain tumors. The study of thyroid hyperplasia may give insight into conditions existing in the gland at the time that the neoplastic transformation occurs.

Proposed Course: I plan to continue the above studies of hyperplasia.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8204-05 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Ultrastructure, Biology, Biochemistry, and Immunology of Mammary Tumor Virus Host-Cell Interaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Gilbert H. Smith, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI

David G. Longfellow, Guest Worker, Laboratory of Molecular Biology, DCBD, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	1.25	2

SUMMARY OF WORK (200 words or less - underline keywords)

Isolation of intracytoplasmic A particles from primary and transplanted mouse mammary tumors. Biochemical, immunologic, ultrastructural, and biologic characterization of the purified particles and comparison of those results with those obtained with similarly purified mouse mammary tumor virus (MMTV).

PHS-6040
(12-75)

Serial No. Z01 CBD 8204-05
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Ultrastructure, Biology, Biochemistry and Immunology of Mammary Tumor Virus - Host Cell Interaction

Previous Serial No.: Z01 CBD 3256-04

Principal Investigator: Gilbert H. Smith, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI

Other Investigators: David G. Longfellow, Guest Worker, Laboratory of Molecular Biology, DCBD, NCI

Man Years: July 1, 1975 through June 30, 1976

Total:	3.25
Professional:	1.25
Other:	2.00

Project Description:

Objectives: The objective of the project is to elucidate the aspects of cell-virus interaction in mouse mammary tumor virus (MMTV) infection and tumorigenesis. The approach includes the isolation and purification of intracytoplasmic A particles (putative MMTV nucleoproteins cores) from primary and transplanted tumors and the purification of MMTV virions from similar sources. Analysis and comparison of the characteristics of each particle is performed in an attempt to establish the ontologic relationship between them within the framework of the replication cycle of the virus; examination and evaluation of MMTV-infected mammary gland explants by biochemical and morphological criteria during induction of differentiated function by hormone treatment (with special emphasis on the relationship of viral replication and expression of differentiated cellular functions.

Major Findings: Intracytoplasmic A particles (CAP) were analyzed by immuno-diffusion and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) before and after controlled enzymatic cleavage with trypsin. An antigen commonly precipitated by anti-CAP, anti-MMTV and monovalent anti-MMTVp28 was demonstrated in purified CAP. However, no SDS-polypeptide migrating at p28 was observed in SDS-PAGE profiles obtained with dissociated CAP. Trypsin cleavage of intact CAP quantitatively converted all CAP SDS-polypeptides to p28 and p15-10 without alteration of the CAP associated antigenic identity with MMTV p28. These results suggested CAP possess polypeptide precursors of MMTV core proteins and that cleavage of CAP structural proteins is a regular feature of the maturational events during virus release. A manuscript was published describing these results. Further investigation

demonstrated the B-mercaptoethanol and dithiothreitol (disulfide bond reducing agents) cause dissociation of intact CAP into separable structural components, only one of which contained MMTV p28 antigenic specificity; analyses of these components by SDS-PAGE showed that higher molecular weight polypeptides CAP p82, p68-60, p37 were present in the particle containing MMTV p28 specificity, whereas only CAP p20-18 was present in the other CAP component. Predigestion with trypsin and subsequent DTT disruption followed by SDS-PAGE analysis showed that p82, p68-60 and p37 were converted to p28; CAP p20-18 to p15-10. A manuscript describing these results is planned.

The nucleic acids associated with purified CAP have been analyzed. A high molecular weight SS DNA was isolated from CAP. Analytical ultracentrifuge, hydroxyapatite chromatography, $S_{20}W$ evaluation and electron microscope measurements have been completed. A manuscript describing this work has been accepted for publication. Confirmation of the presence of DNA in CAP inclusions in situ was obtained by quantitative electron microscope autoradiography and correlative determination of CAP-DNA specific activity following its isolation from the same tumors. A manuscript describing these results was also published.

Evidence for the presence of RNA-DNA heteroduplexes and RNA in total nucleic acid isolates from purified CAP was obtained by analytical ultracentrifugation in CS_2SO4 gradients. Characterization of these molecules by denaturation-renaturation and enzyme susceptibility is planned.

It seems clear on purely immunological grounds that intracytoplasmic A particles represent intracellular MMTV nucleoprotein structures. Evidence that these structures are directly related to MMTV and represent an intermediate step in the development of MMTV nucleoprotein cores was obtained by the quantitative conversion of A particle structural polypeptides by tryptic hydrolysis to polypeptides with molecular weights similar to those in the core of the mature virion without loss of antigenic identity. In addition, this conversion was dependent upon the morphologic integrity of the particle suggesting that the sensitive sites are determined by the three dimensional configuration of the polypeptides in the intact A particle. Additional information regarding the presence of single-stranded DNA, low molecular weight RNA and RNA-DNA hybrids in purified intracytoplasmic A particle preparation requires demonstration by molecular hybridization of the relatedness of these macromolecules to MMTV genomic RNA. Nevertheless the data available at present from this laboratory and from others indicate that intracytoplasmic A particles represent morphologic entities directly related to MMTV which are formed in the cytoplasm as direct precursors to the nucleoprotein cores on the MMTV virions.

A collaborative effort with Dr. Barbara Vonderhaar (NIAMD) has been initiated. Mammary gland explants from MMTV-infected C_3H/He mice are under study. Differentiation of aveolar mammary gland cell functions can be induced by sequential hormonal treatment of the explants with insulin, hydrocortisone and prolactin. These events now may be blocked by various metabolic analogs. Evaluation of these events by biochemical and morphologic

events and the correlation of these phenomena with virus production is planned.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 3. The mouse mammary tumor virus problem has been and is being used as a relevant model for understanding human mammary neoplasia. Yet our knowledge of the intracellular events in MTV infection is essentially nil. The project is designed to provide a base to further our understanding of the intracellular events with the aim of elucidating the replicative cycle of MTV. Subsequently, it is hoped, armed with this information, to specify the tumorigenic influence of the virus.

Proposed Course: To try to specify the tumorigenic influence of the virus.

This project was transferred from Laboratory of Biology to Laboratory of Molecular Biology.

Publications:

Smith, Gilbert H. and Lee, Barbara K.: Mouse Mammary Tumor Virus Polypeptide Precursors in Intracytoplasmic A particles. J. Nat. Cancer Inst. 55: 493-496, 1975.

Gantt, Raymond; Smith, Gilbert H. and Julian, B.T.: Virion - Associated and Cellular RNA Methylase Activity in Normal and Neoplastic Mammary Tissue From Mammary Tumor Virus - Infected and - Uninfected Mice. Cancer Res. 35: 1847-1853, 1975.

Smith, Gilbert H.; Longfellow, D.G., and Nixon, J.N.: Autoradiographic Evidence for the Association of DNA with Intracytoplasmic A Particle Inclusions in situ. J. Nat. Cancer Inst. 55: 1413-1417, 1975.

Wivel, N.A.; Smith, Gilbert H. and Ozer, H.L.: Comparison of Intracytoplasmic A Particles and Intracisternal A Particles. Int. J. Cancer 16: 240-248, 1975.

Smith, Gilbert H.; Kidwell, W.R., and Schweider, W.E.: Cytoplasmic DNA in Rat Corpora Lutein Ceus: Effect of Prolactin on [^{3}H] Thymidine Incorporation. Expt. Cell Res. 96: 321-334, 1975.

Longfellow, D.G. and Smith, Gilbert H.: Further Characterization of Intracytoplasmic A Particle Associated DNA. J. Virol. (in press) March 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8206-03 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Relationship of Genetic and Non-Genetic Factors in Mammary Tumorigenesis in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. Vlahakis, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI

G.H. Smith, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI

H.C. Chopra, Research Biologist, LTVG, NCI

COOPERATING UNITS (if any)

LTVG, NCI

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

SUMMARY OF WORK (200 words or less - underline keywords)

The study of the relationship of genetic factors and non-genetic factors in mammary tumorigenesis in female mice continues. Studies with the different strains of mammary tumor virus (MTV) and the genetically variable inbred mouse strains will help in assessing the relative importance of these factors. Interest is also in the way the various MTV's are transmitted: principally by female or equally well by both sexes. Finally, the strains of mice that develop the interesting hormone dependent mammary gland tumors associated with pregnancy are also being investigated.

Serial No. Z01 CBD 8206-03 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: The Relationship of Genetic and Non-genetic Factors in Mammary Tumorigenesis in Mice

Previous Serial No.: Z01 CBD 3270-02

Principal Investigator: G. Vlahakis, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI

Other Investigators: G.H. Smith, Research Biologist, Laboratory of Molecular Biology, DCBD, NCI
H.C. Chopra, Research Biologist, LTVG, NCI

Cooperating Units: LTVG, NCI

Man Years: July 1, 1975 through June 30, 1976

Total:	0.75
Professional:	0.5
Other:	0.25

Project Description: Objective 1, Approach 4

Objectives: To study the relationship between genetic factors and the milk-borne mammary tumor virus (MTV) in the occurrence of mammary tumors.

Methods and Results: The testing of low oncogenic DD-MTV from mammary tumor strain DD, in strain C3H-A^{vy}fB is complete and the results have been published in JNCI. The viable yellow gene (A^{vy}) which characterizes C3H-A^{vy}fB is an example of the role of genes in mouse tumorigenesis.

Methods and Results: The Haagensen subline of C57BL (formerly designated as C57BL/M) when fostered on strain RIII results in a high incidence of mammary tumors occurring at a late age. Since both strains RIII and DD are of European origin, and both develop hormone dependent mammary gland tumors, and therefore, they may be related, we thought it worthwhile to test DD-mammary tumor virus (MTV) in C57BL/Haag.

Strain C57BL/Haag with DD-MTV was developed by fostering a litter soon after birth on a DD female for only one generation. Approximately 95 C57BL/HaagfDD females in the F_1 through F_4 generations following the foster nursing were produced and maintained as breeders. Results thus far suggest that the mammary tumor incidence and average age at which tumors occur in C57BL/HaagfDD will be similar to C57BL/HaagfRIII and that therefore, RIII-MTV and DD-MTV may

be related. Tumors have occurred in 25 females so far at an average age of approximately 17 months. Histologic study of the mammary tumors shows them to be mainly adenocarcinomas types B and A. Examination by the electron microscope reveals B particles in the tumors providing additional evidence for the propagation and transmission of MTV in C57BL/HaagfDD.

Methods and Results: We now have some results on the fostered strain BALB/cfGR (with GR-mammary tumor virus). The objective here is to compare GR-MTV from the high European mammary tumor strain GR, with C3H-,A-, and DD-MTV's from strains C3H, A and DD respectively. BALB/c females are susceptible to mammary tumors when given MTV through foster nursing on strains that have the milk-borne virus. Approximately 63 BALB/cfGR females have been set aside and they are being maintained as virgins to make possible a comparison with the earlier study, in which it was shown that the three naturally occurring MTV's from C3H, A, and DD ranged from high, to medium, to low ability in the production of mammary tumors only when compared in virgin BALB/c females. To date 3 mammary tumors have occurred in the virgins at an average age of 13 months. Three females were necropsied at an average age of 16 months without mammary tumors. The results thus far indicate that the GR-MTV is like the DD-MTV in potency, and that the incidence and average age of tumor onset in BALB/cfGR virgins will be similar to that for BALB/cfDD virgins. It is expected that the experiment will be nearly complete by the end of the year.

Methods and Results: Since strain BALB/c is widely distributed and is known as a low spontaneous mammary tumor strain, but highly susceptible to the mammary tumor virus (MTV) it is important to know if the strain carries MTV. The incidence of spontaneous mammary tumors in breeding females is approximately 20%, occurring at an average age of about 17 months. Many of the tumors are small adenoacanthomas usually observed at necropsy. However, a few adenocarcinomas do occur that are readily transplantable.

Hageman and Calafat of The Netherlands Cancer Institute, in their experiments believe they have demonstrated that their BALB/c subline does have MTV, which Bentvelzen has named MTV-O for "overlooked".

We have attempted to repeat their experiments using cell-free extracts of mammary glands from old, uninfected breeding females of our strain BALB/c. Cell-free extracts were also prepared from two BALB/c mammary tumor transplants of spontaneous tumors that arose in our strain. The results were completely negative--not a single mammary tumor occurred in the females living well beyond a year, that had received injections of the extracts. Our strain BALB/c continues to be a low spontaneous mammary tumor strain since it was obtained in 1966 from Dr. Andervont's colony. The results with cell-free extracts of old mammary glands and tumor transplants support the view that BALB/c does not have a mammary tumor virus. We are now preparing these results for publication.

Methods and Results: Sabine, Horton and Wicks at the University of Adelaide, in Adelaide, Australia, attributed the high spontaneous mammary and

liver cancer in our strains C3H-A^{vy} and C3H-A^{vy}fB in the United States to the use of cedar shavings in the bedding material. Since their publication in 1973 we have been maintaining some C3H-A^{vy}fB females on low resin pine bedding alone, weaned from mothers most of whom had been on pine. Littermate controls were maintained on bedding consisting of pine plus red cedar shavings.

This study is now complete and the results have been tabulated for publication. Tumor incidences were high in both experimental and control animals. We have no evidence that environmental factors in bedding or diet are contributing to the high incidences of tumors observed in strain C3H-A^{vy}fB which does not have the virulent milk-borne mammary tumor virus. A manuscript is now in preparation and will shortly be submitted as a brief communication.

Methods and Results: Tsubura and his co-workers at Nara Medical College, Nara, Japan, have claimed that like strain GR in which the mammary tumor virus (MTV) is transmitted by either sex, both female and male transmission of MTV occurs in their high mammary tumor substrain DD. In 1959 we started our line of DD, having obtained some breeding animals from Tsubura who had been a guest worker in our laboratory. In reciprocal crosses that we made between strains DD and C57BL, we found that mammary tumors occurred only in the (female DD x male C57BL) F_1 females, and not in the reciprocal (female C57BL x male DD) F_1 females. This was evidence that male transmission of MTV in strain DD probably does not occur. Since strain BALB/c was used in the production of the reciprocal hybrids by Tsubura and his group, we are repeating their work using our strain BALB/c.

Thirty-nine (female DD x male BALB/c) F_1 females and 40 (female BALB/c x male DD) F_1 females have been produced and they are being maintained as virgins. To date 3 mammary tumors have occurred in the (female DD x male BALB/c) F_1 group at an average age of 10.3 months. We should have more definite information later this year of whether or not male transmission of MTV occurs in DD when it is outcrossed to the susceptible BALB/c strain.

Methods and Results: In 1971 we published a paper on the spontaneous cholangiomas occurring in our high hepatoma strain C3H-A^{vy}fB and in their hybrids. Later Turusov et al., who believe the tumors should be referred to as hepatoblastomas, and not cholangiomas, found an increase in their occurrence among mice receiving the pesticide DDT in the diet. We have been feeding DDT to some of our C3H-A^{vy}fB, both males and females, to see whether or not it will increase the incidence of cholangiomas. These interesting liver tumors have always been observed in association with hepatomas, and since strain C3H-A^{vy}fB is highly susceptible to the development of hepatomas in both sexes, it seemed worthwhile to test the effect of DDT in this strain. A group of control animals has also been included in this study feeding them our normal standard food.

All of the DDT and most of the control animals have now been necropsied, and all had hepatomas but the occurrence of cholangiomas will be similar in both groups. A histologic study is now being done on the hepatomas in the

DDT and control animals and the data will be tabulated and analyzed for possible publication.

Again this year, collaborative studies on mammary tumors and hepatomas with other investigators both here and outside the NIH, are continuing. Our contribution of valuable mammary tumor and hepatoma mouse strains, and our knowledge of the biology of these neoplasms make such studies desirable, and therefore are actively being pursued. One in particular, with Dr. Rob Michalides of the Dutch Cancer Institute in the Netherlands, and Dr. Jeffrey Schlom of the NCI, on molecular hybridization studies utilizing mouse MTV, is now complete and has been submitted for publication. Also some time has been spent with other research workers both here and outside of the NIH, helping them to develop their own programs. This has consisted of discussions and of making available to them specific biological material for their own research requirements. Time has also been spent talking with students and others who visit the laboratories at NIH as part of their learning experience.

Significance to Cancer Research and the Program of the Institute: The occurrence of mammary tumors in mice depend on the interplay of many factors: the genotype of the animal, the mammary tumor virus, hormones, environment, etc. Studies of mammary tumorigenesis in mice need to be done so that eventually mechanisms of interaction between the various factors will be understood and therefore, quite possibly have relevance for the disease process in man.

Proposed Course: The study of the fostered strain C57BL/HaagfDD (with mammary tumor virus from strain DD) will be complete by the end of the year and the data will be tabulated and analyzed for publication.

This project was transferred from the Laboratory of Biology to the Laboratory of Molecular Biology.

Publications:

Vlahakis, G.: Response of C3H-A^{vy}fB Female Mice to a Low Oncogenic, Milk-borne Mammary Tumor Virus. J. Nat. Cancer Inst. 55: 1217-1218, 1975.

Chopra, Harish C., Woodside, Norman and Vlahakis, George: New Morphological Variants of Mouse Mammary Tumor Virus in High Tumor Strains of Mice. Microbios 13: 41-50, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8203-02 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Induction of Oncornavirus Expression in Mammalian Placental Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kurt Stromberg, Sr. Staff Investigator, Laboratory of Molecular Biology, Gene Regulation Section, DCBD, NCI

K. V. Speeg, Research Associate, Laboratory of Molecular Biology, Gene Regulation Section, DCBD, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Trophoblast cells from the placental villi represent the only type of human cell in which spontaneously budding type-C concornaviral particles have been seen with regularity by electron microscopy. This observation implies that the structural gene for complete type-C particle synthesis is present in at least some human cells, and that the perhaps generally prevailing pattern of suppression of expression by regulatory gene(s) is not absolute. The demonstration in primary C57BL/6 mouse brain cells that, following fusion to a permissive human line (V_{a2}), expression of mouse type-C particles occurred in hybrid cells suggests that murine regulatory gene(s) were lost, presumably through cell fusion and resulting chromosome segregation (P.N.A.S. 71: 1695-1700, 1974). This conceptual and experimental background has led us to plan to apply somatic cell hybridization to the problem of oncornaviral induction in human placental cells. Over the past nine months we have traced the culture morphology, longevity of passage in culture, duration of expression of chorionic gonadotropin (HCG), and response to chemical oncornaviral inducing agents (e.g. iododeoxyuridine), of human placental cells in primary culture.

Serial No. ZO1 CBD 8203-02 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Induction of Oncornavirus Expression in Mammalian Placental Cells

Previous Serial No.: ZO1 CBD 03271-01 LBGY

Principal Investigators: Kurt Stromberg and K. V. Speeg, Laboratory of Molecular Biology, Gene Regulation Section

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Summary of Work: Trophoblast cells from the placental villi represent the only type of human cell in which spontaneously budding type-C oncornaviral particles have been seen with regularity by electron microscopy. This observation implies that the structural gene for complete type-C particle synthesis is present in at least some human cells, and that the perhaps generally prevailing pattern of suppression of expression by regulatory gene(s) is not absolute. The demonstration in primary C57BL/6 mouse brain cells that, following fusion to a permissive human line (V_{a2}), expression of mouse type-C particles occurred in hybrid cells suggests that murine regulatory gene(s) were lost, presumably through cell fusion and resulting chromosome segregation (P.N.A.S. 71: 1695-1700, 1974). This conceptual and experimental background has led us to plan to apply somatic cell hybridization to the problem of oncornaviral induction in human placental cells. Over the past nine months we have traced the culture morphology, longevity of passage in culture, duration of expression of chorionic gonadotropin (HCG), and response to chemical oncornaviral inducing agents (e.g. iododeoxyuridine), of human placental cells in primary culture.

This project was transferred from the Lab. of Bio. to the Lab. of Mol. Bio.

Objectives:

I. Development of methods of primary culture of trophoblast cells from first trimester human placentas.

II. Characterization of pattern of growth and differentiation (HCG? endogenous oncornaviral? expression) of resulting primary placental cell strains.

III. Induction by cell fusion and chromosome segregation of oncornavirus expression in human placental cells.

Methods Employed: Sequential, brief exposures of unminced human placental villi to EDTA-trysin solutions released single cells with HCG activity that was measured by competition radioimmunoassay. Oncornavirus presence in culture fluid is detected by assay for primate oncornaviral p30 polypeptides and for exogenous DNA polymerase activity using synthetic template-primer combinations of poly rA: oligodt or poly Cm: oligo dG. A human diploid, HGPRT-deficient, fibroblast line (V_{a2}) is used as a permissive parental line in cell fusion studies with primary placental cells.

Major Findings: Two continuous lines of human choriocarcinoma cells (JAR and BeWo), as well as several lines to be used in cell fusion studies as permissive parental lines, did not yield evidence of oncornavirus expression following exposure to iododeoxyuridine and dexamethasone when followed for 4 months by initially daily and then biweekly assay of DNA polymerase activity in the high speed pellet from culture fluids. Ten of twelve strains of primary placental cells which each originated from separate placental specimens were similarly treated and examined. Intermittent evidence of DNA polymerase activity was determined to be cellular (terminal transferase), not oncornaviral (reverse transcriptase), in origin. The placental cell strains were each developed from primary cultures in which colonies of cells of epithelial morphology were twice isolated by penicylinders prior to subculture. Detectable HCG activity continued into the third passage. Senescence of the strains occurs between passage 12 and 16.

Significance for Cancer Research and the Program of the Institute: The relevance of the oncornaviral animal model to human cancer will be in question until a bonafide oncornavirus of human origin is growing in cell culture in adequate yield. Only then can appropriate detection techniques, such as radioimmune precipitation assays for viral polypeptides, be developed to correlate oncornaviral expression with neoplastic growth in man. This project is devoted to this aim, namely, to gain human oncornaviral expression in cell culture.

Proposed Course: Selected frozen passages of placental cell strains, in which the approximate passage of senescence has been established, have been reconstituted. These are being fused to V_{a2} and grown in HAT medium. The unfused parental cells will die by senescence and HGPRT deficiency, respectively. Hybrid cells will be examined over extended periods in culture for evidence of oncornaviral expression.

Primary culture of placental cells from New World monkeys, as well as Gibbon ape and Rhesus monkeys, is contemplated. If oncornavirus expression following chemical induction is not detected, the cell strains will similarly be subjected to somatic cell hybridization as a technique to induce primate endogenous oncornavirus production.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 8205-02 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Modulation of Differentiated Functions in Choriocarcinoma Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kermit V. Speeg, Research Associate, Laboratory of Molecular Biology, DCBD, NCI
Kurt Stromberg, Sr. Staff Investigator, Laboratory of Molecular Biology, DCBD, NCI

COOPERATING UNITS (if any)
NIAMDD, CE
NCI, LB

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Human choriocarcinoma is being maintained in tissue culture and the modulation of two products (chorionic gonadotropin and placental alkaline phosphatase) which are tumor markers is being examined. The ability of BCG-activated macrophages to kill human choriocarcinoma is also being examined.

PHS-6040

Serial No. ZO1 CBD 8205-02
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Modulation of Differentiated Functions in Choriocarcinoma Cells in Culture

Previous Serial Number: ZO1 CBD 03272-01

Principal Investigator: K.V. Speeg

Other Investigators: Kurt Stromberg

Cooperating Units: NIAMDD, CE
NCI, LB

Man Years: July 1, 1975 through June 30, 1976

Total:	3.5
Professional:	2
Others:	1.5

Project Description:

Objective 1: To maintain human choriocarcinoma cells in tissue culture and to study their growth characteristics.

Findings: A) Under certain circumstances these cells will grow in serum-free media allowing experiments under defined conditions to be carried out. B) Fetal serum enhances rate of cell division, results in less HCG/mg protein and causes smaller and more numerous cells than newborn calf serum.

Objective 2: To study in detail modulation of two cell products - human chorionic gonadotropin and placental alkaline phosphatase.

Findings: A) HCG is a protein hormone induced by CAMP (or its analogs or PDE inhibitors) by a process dependent on protein synthesis and apparently independent of RNA or DNA synthesis. In fact inhibitors of both RNA and DNA synthesis result in induction of HCG.

These cells lack peak I binding protein - protein kinase (DEAE cellulose method of Gilman) under certain conditions of growth whereas normal placenta has both peak I and II. A method to induce synthesis of peak I has been found and the presence or absence of this peak is being investigated with respect to rate of HCG synthesis.

B) Alkaline phosphatase is a membrane bound glycoprotein whose function is poorly understood. It's activity can be induced with halogenated pyrimidines or butyrate. Choriocarcinoma in tissue culture contains 2 forms - one, the expected fetal isozyme which is heat stabile and shows a specific pattern of amino acid inhibition and another isozyme which resembles a liver form being heat labile and inhibited in a different pattern by several amino acids. Gene regulation is being studied by modulation of DNA and RNA synthesis.

Objective 3: To set up and test the Hibb's BCG-activated macrophage system and ascertain whether or not choriocarcinoma cells are recognized as malignant.

Finding: The Hibb's system has been set up and tested. Because normal trophoblast shows rapid growth, tissue invasion, blood supply organization and ability to metastiasize we wished to test whether the macrophage system could recognize choriocarcinoma as malignant. It could not and these cells were treated as normal. The basis for this is being explored.

Significance to Cancer Research and the Program of the Institute: Objective 1, Approach 4; Objective 3, Approach 2; Objective 4, Approach 1 and Objective 5, Approach 4.

Knowledge of the basic biology of a particular kind of human cancer is a necessary prerequisite to understanding its etiology and control. Choriocarcinoma in tissue culture represents a model system to study human malignancy. The normal counterpart of these cells (placenta) is said to possess the ability to grow rapidly, organize blood supply, invade normal tissue and metastasize. It would be highly desirable to know how this happens but this information is largely unavailable. It is difficult to study experimentally for obvious ethical reasons. Choriocarcinoma cells retain many of the normal features of placenta but have the added advantage of growing in tissue culture and being useful for experimentation. Choriocarcinoma cells possess two glycoproteins (HCG and placental alkaline phosphatase) currently being used as cancer markers with both diagnostic and prognostic value. However, those factors responsible for modulation of the markers are basically unknown but ultimately essential for the markers to be useful clinically. They are easily studied in this tissue culture system.

Moreover, the effect and mode of action of various chemotherapeutic and immunotherapeutic agents may also be assessed with these cells. It represents, therefore, a microcosm in which to conduct the war on cancer.

Proposed Course: It is planned that much of the work centering around modulation of alkaline phosphatase and HCG will be completed to coincide with the departure from the PHS of several of the investigators. However, study of these modulation parameters will continue and be extended to a second line of choriocarcinoma by those remaining.

This project was transferred from Lab. of Bio. to the Lab. of Mol. Biol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8202-07 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structural Characterization of Avian Myeloblastosis Virus (AMV)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kurt Stromberg, Sr. Staff Investigator, Gene Regulation Section, LMB, DCBD, NCI
A. Deeney and G.S. Beaudreau,

COOPERATING UNITS (if any)

Dept. of Agricultural Chemistry, Oregon State University

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
Bethesda, Maryland 20014 National Cancer Institute, National Institutes of Health

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.00

SUMMARY OF WORK (200 words or less - underline keywords)

Rapid, simple, and quantitative techniques have been developed for separation of the envelope, core, and RNP components of a representative type-C oncornavirus. The most recent subject in a series of biochemical studies of these subcomponents of AMV has been to probe for the presence of DNA within the internal core structure by measuring priming activity. Although core preparation retained only 10% of the viral protein, they contained over 70% of the DNA found in the companion virion preparations. Even if the DNA is of cellular origin, the necessary excision of DNA and packaging within the core may reflect on important action of the tumor virus on the host cell.

Serial No. ZO1 CBD 8202-07 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Structural Characterization of Core and Ribonucleoproteins (RNP) Components of Avian Myeloblastosis Virus (AMV)

Previous Serial No.: ZO1 CBD 03254-06

Principal Investigator: Kurt Stromberg, Sr. Staff Investigator, Gene Regulation Section, LMB, DCBD, NCI
A. Deeney and G.S. Beaudreau, Dept. of Agricultural Chemistry, Oregon State University

Cooperating Units: Dept. of Agricultural Chemistry, Oregon State University

Man Years:

Total:	0.25
Professional:	0.25
Other:	0.00

Project Description:

Summary of Work: Rapid, simple, and quantitative techniques have been developed for separation of the envelope, core, and RNP components of a representative type-C RNA tumor virus. The most recent subject in a series of biochemical characterizations of these subcomponents of AMV has been to probe for the presence of DNA within the internal core structure of the virion. If present, it would imply a possibly significant role for the viral DNA in the life cycle of the virus.

Objective: The objective was to compare the relative amount, if any, of DNA in AMV cores with the amount, if any, found in purified AMV virions. Detection of DNA was determined by measuring priming activity of the putative DNA over a range of proportionality between DNA primer and [^{3}H] DNA synthesis in the presence of purified reverse transcriptase from AMV. As expected from earlier studies, preparations of purified virus that were treated with nonionic detergent to remove the outer viral envelope retained only about 10% of the viral protein. However, these core preparations contained over 70% of the DNA found in the companion virion preparations. Whatever the source of the DNA, it appears to be associated also with the core component. Even if the DNA is of cellular origin, the necessary excision of DNA and packaging in the viral core component is an intriguing problem and may reflect an important action of the tumor virus on the host cell.

Significance to Cancer Research and the Program of the Institute:

National Cancer Plan: Objective 5, Approach 4
The possible association of type-C RNA tumor viruses in human cancer dictate that the structural, biochemical, and enzymatic characteristics of animal type-C viruses be determined as a prerequisite to detection of similar viral-like characteristics in human fluids and precancerous tissues.

Proposed Course: The presence of small amounts of DNA in AMV core preparations was unexpected. Whether this represents trapping of extraneous host cell DNA during virus maturation, or is pertinent to viral replication can be evaluated by experiments which determine if this core-associated DNA hybridizes to viral RNA and not to host DNA.

This project was transferred from Lab. of Biology to the Lab. of Molecular Biology.

Publications:

Deeney, A. O'C., Stromberg, K., and Beaudreau, G.S.: Identification of DNA in the core component of avian myeloblastosis virus. Biochim. Biophys. Acta 432: 281-291, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 8200-07 LMBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Gene Regulation of Protein Turnover and Its Relationship to Tumorigenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Harold A. Hoffman, Research Geneticist, Laboratory of Molecular Biology, DCBD, NCI
Charles K. Grieshaber, Sr. Staff Fellow, Laboratory of Molecular Biology, DCBD, NCI
Warren Evans (Laboratory of Biochemistry, NCI)
Roberta Van Nei (Netherlands Cancer Institute)
E. A. Boyse (Sloan Kettering Institute)
S. Nandi (Cancer Research Laboratory, Univ. of California, Berkeley)
M. R. Banerjee (Tumor Biology Laboratory)

COOPERATING UNITS (if any)
Office of Program Resources and Logistics, DCCP, NCI (Supplies rabbit anti-MTV serum)
Transplantation Immunology Branch, NIAID (Supplied allotypic histocompatibility typing sera)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

This study utilizes the techniques of quantitative genetics, biochemistry and immunochemistry. Female first generation backcross mice are tested for the presence or absence of viral group specific antigen by immunofluorescent absorption and for the allelic distribution of biochemical marker genes on 11 different chromosomes. The data are anlyzed by standard genetic linkage programs.

Serial No. ZO1 CBD 8200-07 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Gene Regulation of Protein Turnover and Its Relationship to Tumorigenesis

Previous Serial No.: ZO1 CBD 03208-06 LBGY

Principal Investigator: Harold A. Hoffman

Other Investigators: Charles K. Grieshaber
Warren Evans (Laboratory of Biochemistry, NCI)
Roberta van Nei (Netherlands Cancer Institute)
E. A. Boyse (Sloan Kettering Institute)
S. Nandi (Cancer Research Laboratory, Univ. of California, Berkeley)
M. R. Banerjee (Tumor Biology Laboratory, Univ. of Nebraska)

Cooperating Units: Office of Program Resources and Logistics, DCCP, NCI (Supplies rabbit anti-MTV serum)
Transplantation Immunology Branch, NIAID (Supplied allotypic histocompatibility typing sera)

Man Years:

Total:	5
Professional:	2
Others:	3

Project Description: Objective 3. Approach 3

Objectives: To study by methods appropriate to biochemical and quantitative genetics the gene regulation of protein synthesis and degradation in the mouse and other mammals and to determine the genetic and non-genetic relationships between protein metabolism and tumorigenesis. To determine the primary structure of the normal protein and the aberrant or mutant protein in order to define the gene or genes controlling their production and to determine the time of gene action during the process of neoplastic transformation.

Methods: In these investigations mice from 18 inbred strains and substrains, which represent at least six different independent lines of

known genetic origin, are used as sources of proteins and tumors, and for the production of allotypic antiserum. New Zealand white rabbits of known immunoglobulin type are used for the production of heterologous antiserum, and non-human primates of the order anthropoid are used as a source of protein.

Immunochemical methods, agar-gel double diffusion, immuno-electrophoresis, and immunofluorescent absorption, are used in the detection and characterization of different proteins. Physical and chemical techniques of gel electrophoresis and chromatography are used to isolate and classify different proteins. The fine structure and minute differences between closely related proteins are determined by peptide map analysis (fingerprints) and amino acid analysis.

Major Findings:

Inheritance of tissue and erythrocyte catalase in inbred mice. Starch-gel electrophoresis of kidney catalase in inbred strains C3H and C57BL/6, their F_1 hybrid, and first and second backcross generations demonstrated that single-component (Type A) versus multiple-component (Type B) electrophoretic patterns are controlled by a single locus. The Type A electrophoretic pattern is dominant. The data indicate that the segregating genetic factor determines a specific substance in the Type A kidney which affects the electrophoretic mobility of catalase. A comparison of the F_1 hybrid enzyme with C3H and C57BL/6 enzyme showed that the alteration of electrophoretic mobility is the result of post-translational modification of the catalase molecule.

Two-factor linkage analysis indicated that the kidney catalase electrophoretic pattern locus is 4.2 $\pm$ 2.3 map units from the H-2D end of the H-2 complex on chromosome 17.

Experiments defining the mechanisms governing catalase levels in BALB/c liver (191 EU/mg) and kidney (96 EU/mg) indicate that tissue specificity is due to differences in the rate of synthesis of catalase rather than rate of degredation or the turnover number, K_1'.

Inheritance of the expression of MTV group specific (gs) antigens.

Experiments in collaboration with Dr. van Nei have uncovered a genetic factor associated with the presence and absence of NIV viral antigen. Linkage analysis indicates that this factor is on chromosome 7 located somewhere between the structural gene for glucose phosphage isomerase and the structural gene for the beta chain of hemoglobin.

In collaboration with Dr. Banerjee, we have successfully induced in mammary glands from 4 week old virgin mice in organ culture hormone-dependent tumors. The hormone regimen used was insulin, growth hormone, estrogen, progesterone, aldosterone, and prolactin. In vivo, hormone-dependent tumors usually appear at the 15th day of gestation and regress around the third day of lactation.

Significance for Cancer Research: (NCP Objective 3, Approach 3). This study provides an animal model for the determination of genetic and non-genetic factors for human breast cancer.

Proposed Course of Project: Three-factor linkage analysis to determine the kidney catalase electrophoretic factor location in relation to the H-2D end of the H-2 complex and the thymus leukemia antigen locus.

To continue experiments on the organ culture of murine mammary glands. Determine the hormone regimens for the appearance and regression of the hormone-dependent tumors and to develop the system for hormone receptor analysis.

Associated Activities: Assistant Project Officer for 5 contracts awarded by the experimental Biology Committee of the Breast Cancer Task Force. The projects are in the areas of the role of hormones in tumorigenesis and the characterization of mammary gland epithelial cells.

Participation on the Board of Editors of the Journal of the National Cancer Institute as an Associate Editor.

This project was transferred from the Lab. of Bio. to the Lab. of Mol. Bio.

Publications:

Grieshaber, C. K. and Hoffman, H. A.: Genetic regulation of the expression of catalase activity in murine red blood cells. Biochem. Genet. 13: 447-456, 1975.

Hoffman, H. A. and Grieshaber, C. K.: Genetic studies of murine catalase: Regulation of multiple molecular forms of kidney catalase. Biochem. Genet. in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 8201-04 LMBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Regulation of Enzyme Expression in Normal Differentiating and Neoplastic Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Charles K. Grieshaber, Ph.D., Sr. Staff Fellow, Laboratory of Molecular Biology, DCBD, NCI
Harold A. Hoffman, Ph.D., Research Geneticist, Laboratory of Molecular Biology, DCBD, NCI
Warren H. Evans, Ph.D., Research Chemist, Laboratory of Biochemistry, DCBD, NCI
M. R. Banerjee, Tumor Biology Laboratory, Univ. of Nebraska
E. A. Boyse, Sloan Kettering Institute

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NCI
Transplantation Immunology Branch, NIAID (Supplied allotypic H-2 typing sera)

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Gene Regulation Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

The differentiation of enzyme-expression in normal and neoplastic mammary glands and bone marrow cells is being studied in order to define those processes which regulate the appearance or loss of activity and, thus, modify cellular function. One specific case currently under investigation is the mechanisms by which polyamines participate in overall cellular metabolism; another is the effect of hormones on cellular reactions during and after neoplastic transformation.

Serial No. ZO1 CBD 8201-04 LMBGY
1. Laboratory of Molecular Biology
2. Gene Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: Regulation of Enzyme Expression in Normal Differentiating and Neoplastic Tissue

Previous Serial No.: ZO1 CBD 3268-03 LBGY

Principal Investigator: Charles K. Grieshaber

Other Investigator: Harold A. Hoffman
Warren H. Evans (Laboratory of Biochemistry, NCI)
M. R. Banerjee (Tumor Biology Laboratory, Univ. of Nebraska)
E. A. Boyse (Sloan Kettering Institute)

Cooperating Units:

Laboratory of Biochemistry, NCI
Transplantation Immunology Branch, NIAID (Supplied allotypic H-2 typing sera)

Man Years:

Total:	3
Professional:	1.5
Others:	1.5

Project Description:

Objectives: To study the mechanisms, both biochemical and genetic, involved in regulating the synthesis of those specialized proteins and enzymes which determine whether a cell or tissue is capable of functioning in the differentiated state. Since the operational definition of the differentiated state implies the capacity for the synthesis of specialized, and often specific, proteins, it is our interest to study these processes with respect to deviations from normal during tumorigenesis. It is the basic goal of this project, then, to describe similarities and dissimilarities between normal and tumor cells with respect to the acquisition of specialized proteins.

Methods: Tissues such as bone marrow and peripheral blood cells, liver and hepatomas, kidney, mammary gland and mammary tumors are used as the source of enzymes and other proteins upon which appropriate physical,

biochemical, genetic, and immunochemical analyses are carried out. The enzymes currently under investigation are catalase, myeloperoxidase, arginase, ornithine decarboxylase and S-ardenosyl L-methionine decarboxylase (putrescine and spermidine activated) -- those enzymes in which extracellular regulatory factors can influence modulation. Mice from eight inbred strains and their F_1 hybrids and strain 13 guinea pigs are used as the source of tissues.

Major Findings:

1. In the continuing study designed to describe the biochemical and genetic regulation of the expression of catalase activity in liver, kidney, and RBC's of inbred mice we have found that the turnover number or specific activity of the enzyme is relatively constant in tissues within the same strain. For example, the liver and erythrocyte catalase specific activity (k_1') in BALB/c mice is 4.0-4.1 x 10^7/m/sec, while the k_1' for C57Bl and C57BL/6 mice is 2.3-2.5 x 10^7/m/sec. We have previously demonstrated that the Ce-1 locus in the erythrocyte is the gene responsible for k_1' in the RBC. At present we have some data which suggests that there may be a similar allele in the solid tissues although we only have circumstantial evidence in this regard.

We also have data which show that the intrastrain rates of catalase synthesis in the kidney and liver are different, whereas the rate of enzyme degradation is similar. For example, the K_D of BALB/c kidney and liver catalase is 0.01, while the liver K_s is 2.40 and that of kidney is 0.94. However, interstrain variation in K_s and K_D does exist. While intrastrain K_D is not different, interstrain K_D does vary, eg. K_D of C57BL/6 liver and kidney is twice that of C57BL. Our preliminary analysis of the data suggests at least 3 loci involved in the expression of catalase activity in solid tissues: a) a locus regulation k_1', b) a locus controlling K_D, and c) a locus governing K_s.

2. Studies on polyamine levels in strain 13 Guinea Pig bone marrow granulocytes were carried out to determine the significance of the very high levels of ornithine decarboxylase (ODC) levels in the immature cells. These cells, separated by our Ficoll density gradient centrifugation technique, contain 10-12 times the ODC activity as do mature polymorphonuclear leukocytes. The immature granulocyte has a significantly higher spermidine and spermine concentration compared to the mature granulocyte. The putrescine concentration is not different in the two cell populations.

Intra-peritoneal injection of Shellfish glycogen leads to a depletion of the mature elements in the bone marrow. Over a period of time post-injection compartment proliferates in response to the stimulus. We have found that prior to an increase in DNA synthesis, ODC and SAMDC enzyme activities increase 12 and 4 fold, respectively, in the myeloblasts. It is interesting to note, however, that the concentrations of putrescine and

spermidine are essentially unchanged. In order to test whether the ODC and SAMDC activities represented an increase in products, the incorporation of ^{14}C-labelled ornithine into polyamines was studied. The data show that the level of labelling in the polyamines was consistent with the level of enzyme activity.

The presence of radioactive putrescine was elevated 12 fold in immature granulocytes compared to mature cells, while spermidine label was elevated 4 fold. The radioactivities of putrescine and spermidine are also significantly elevated following intraperitoneal glycogen injection. We have concluded that guinea pig bone marrow cells appear to maintain a relatively constant polyamine pool regardless of the physiological state of the animal. Upon stimulation by glycogen, the cells of the bone marrow synthesize polyamines at a rapid rate yet the pool is unchanged presumably due to an increased rate of removal from the pool.

We have commenced experiments designed to use ODC enzyme activity levels in immature bone marrow cells as an assay for granulopoietic factors. To date we have found that serum from leukopenic guinea pigs stimulates the ODC activity in normal bone marrow cells in culture to a level 3-4 times normal.

3. The metabolism of polyamines in relation to leukemia is being studied in an acute granulocytic leukemia induced in the DBA/2 mouse. This particular leukemia is carried in the ascites form and has the morphological characteristics of leukemic blast-like cells. The polyamine concentration and synthesizing enzyme activities are present in levels associated with rapid growth. These levels are 25 times (250 EU/10^7 cells) the level found in normal murine bone marrow. It is hoped that inhibition of polyamine synthesis might lead to differentiation of these cells.

4. Studies on the hormone dependent tumor of the GR mammary gland have led us to the finding that organ cultured glands can and do grow out plaques which are indistinguishable from the in vivo lesion. These studies are noteworthy because the entire scheme of tumorigenesis from growth to regression can be observed in vitro. Since these lesions are hormone dependent, the actual hormonal regulation of their growth and concomitant intracellular events can be studied in greater detail.

Significance for Cancer Research: NCP Objective 3. Approach 3. This project provides a model system in tissues from animals of known genetic composition for the study of protein and enzyme regulation and interaction during neoplastic transformation. This system could have considerable significance in understanding the general processes of differentiation and tumorigenesis since recognition of the several potential points in the regulation of key enzymes will provide an accurate description of metabolic sequences and biochemical lesions.

This project was transferred from the Lab. of Bio. to the Lab. of Mol. Bio.

Proposed Course:

1. To identify and link the loci responsible for the overall expression of catalase activity in the mouse.

a.) Study catalase synthesis and degradation in liver and kidneys of F_1 hybrids whose parental values are known to differ.

b.) Determination of the specific activity of the catalase enzyme in liver and kidney of C57BL, NBL and DAB/2 inbred strains and F_1 hybrids.

2. Using the ODC activity as assay for granulopoietic factors.

a.) Fractionation of serum from leukopenic guinea pigs to determine the serum substances responsible for the observed effect.

3. Culture GR Mammary Glands in an effort to identify the biochemistry involved in hormone dependent lesion formation.

Publications:

Grieshaber, G. K. and Hoffman, H. A.: Genetic regulation of the expression of catalase activity in murine red blood cells. Biochem. Genetics 13: 447-455, 1975.

Hoffman, H. A. and Grieshaber, C. K.: Genetic studies of murine catalase: Regulation of multiple molecular forms of kidney catalase. Biochem. Genetics, in press, 1976.

LABORATORY OF PATHOPHYSIOLOGY
SUMMARY STATEMENT
ANNUAL REPORT
July 1, 1975 through June 30, 1976

This has been the first complete year that the Laboratory of Pathophysiology has worked in its new quarters and under its new organizational structure. The reorganization of the laboratory, however, is not yet completed. Dr. Pinto da Silva, who recently joined the laboratory, is now setting up facilities to study cell membrane biology. The Laboratory of Physiology was abolished and its investigators have been transferred to the Laboratory of Pathophysiology. The transfer has been completed on paper and is slowly occurring in reality. This summary includes the work of the Laboratory of Pathophysiology as well as that of the former Laboratory of Physiology.

Present research focuses on the following problems:

(1) The regression process in mammary tumors after hormonal deprivation of the host.

(2) The role of poly(ADP-Ribose) in cell cycle regulation.

(3) The behavior of ribosomes and mRNAs in resting and growing human lymphocytes.

(4) The role of extranuclear DNA and nucleotide metabolism in normal and neoplastic tissues.

(5) The physiological and behavioral control of food and water intake and its relation to cancer cachexia.

(6) The effects of ionizing radiations on tissue components and the whole body.

1) Regression Process in Mammary Tumors:

Over the past few years we showed that as the host is deprived of hormones, single cells in the solid tumor start regressing, apparently at random. The number of regressing cells increases with time and the event does not appear to be determined by systemic metabolic alterations or by an immunological type of tissue rejection. Regression occurs as a cell-centered event, with autodigestion as the most prominent feature. Dr. Rouleau endeavored to establish whether autodigestion was the cause of cell destruction or a consequence of events which make the cell components prone to lysis. In favor of the second hypothesis, he showed that within 24 hrs from hormone withdrawal the cytosol proteins become more susceptible to lytic enzymes. He is now expanding this observation.

During regression of DMBA and MTW9 mammary carcinomas, Dr. Bodwin observed a reduction in G6PD activity and demonstrated that this reduction was due to a disappearance of the microsomal G6PD isoenzyme. A similar observation was made after injecting the host with dibutyryl cAMP, which is also able to produce growth arrest of both tumor types. The study of the interrelationship between hormone withdrawal and cAMP behavior during tumor regression is being expanded. Dr. Cho-Chung observed a reduction of estrogen-binding activity in nuclei of DMBA tumors of ovariectomized animals. In the nuclei, however, a sharp increase of cAMP binding occurred, as well as an elevation of adenylate cyclase and cAMP-phosphodiesterase. Injection of estradiol-17β reversed the changes and the tumor resumed growth. As the binding capacity increased in the nuclei, it decreased in the cytosol. Protein kinase activity behaved similarly. This, however, occurred only in the tumors which regressed under dibutyryl cAMP treatment. Moreover, the cAMP binding proteins of these regressing tumors showed a higher stability to heat and pH changes. The study of nuclear translocation of cAMP+binding protein from the cytosol into the nuclei and its role in the regression process will be continued.

Dr. Qasba is approaching the study of the tumor regression process by analyzing the capacity of mammary cells to synthesise cascein and α-lactalbumin under the action of several hormones. He has purified rat casein and α-lactalbumin and has found two molecular species of rat α-lactalbumin with different K_m for galactosyl transferase. He has measured the production of both proteins by neoplastic cells in vivo and in vitro and is now attempting to define the optimal culture conditions for production. With Dr. Chakrabartty, he has isolated and purified mRNA from rat lactating glands and has prepared specific antisera. Efficient translation of this mRNA was obtained using the wheat-germ system. Now the role of hormonal stimulation in the synthesis of this mRNA will be studied and the behavior of the cell synthesizing capacity will be utilized as a probe to elucidate the events occurring during tumor regression.

In trying to develop an in vitro system to mimic the regression of solid tumors, Dr. Knazek has cultivated a human mammary carcinoma (MCF7) on an artificial capillary network to constitute a solid tissue mass with formation of pseudotubuli and desmosomes between adjacent cells. Removal of estrogen, insulin, and human placental lactogen from the media produced a 3-fold decrease in ^{3}H-thymidine uptake and an apparent disorder of the cellular organization. An in vitro model of solid tumor regression however, is not yet available.

2) The Role of Poly(ADP-Ribose) in Cell Cycle Regulation:

In previous experiments Dr. Kidwell demonstrated that nuclei of eukaryotic cells polymerized the ADP-Ribose moiety of NAD through a 1'-2'-ribose-ribose linkage to form poly(ADP-Ribose). The reaction occurred at the S/G_2 phase of the cell cycle and the polymer was bound to chromosomal proteins. In human cell lines (HeLa and HBL-100) he has now found that poly(ADP-Ribose) electrophoresed with HI histone as in rodents, but with a protein band which in SDS-urea gels splits into two species of estimated MW 27500 and 15700

daltons. Dr. Kidwell has developed a radioimmunoassay for quantitating poly (ADP-Ribose) and has estimated that during the cell cycle at the S/G_2 phase, there is about 10^6 copies of the oligomer per cell or about 1 oligomer chain per copy of acceptor protein. When treated with 5'methylnicotinamide, which inhibits poly(ADP-Ribose) polymerase, the cells became arrested at the S/G_2 phase and the poly(ADP-Ribose) quantity was reduced to less than half.

As an expansion of this work Dr. Lorimer III observed that poly(ADP-Ribose) polymerase decreased with the onset of tumor regression and increased again with tumor regrowth. The same observation was made for MCF7 human mammary carcinoma cells in vitro during log-phase growth as contrasted with the stationary phase. Rat mammary tumor nuclei incubated with ^{3}H-NAD produced labeled poly(ADP-Ribose) which is attached to histone. The H1 protein was extracted, electrophoresed on SDS-urea gels and a radioactive protein band separated from HI. When poly(ADP-Ribose) synthesis was inhibited, the protein was absent from the PCA extract. The role of tumor regression, due to hormone deprivation or antiestrogens, in the synthesis of the protein band to poly(ADP-Ribose) is now being studied.

In the case of PCA extraction and purification of H1 histone from HeLa cells Dr. Stone observed that 18% TCA precipitated an adenylated protein with an approximate MW of 10,000 daltons. He also found that 70% of the adenylated protein is in the nuclei; however, it is not made by the nuclei but is probably concentrated from the cytoplasm. Adenylation of the protein is almost instantaneous and it is maintained as long as ATP is present. In whole cells there are about $6x10^4$ acceptor protein molecules which are formed throughout S, G_2, and M phases. A major effort is underway to characterize the adenylated protein and to understand its importance in the cell cycle and its relation, if any, with poly(ADP-Ribose) + protein complex.

3) Behavior of Ribosomes and mRNA in Resting and Growing Human Lymphocytes:

In previous years Drs. Cooper and Berger demonstrated that human lymphocytes in a resting state maintain about 70% of their cytoplasmic ribosomes as single 80S monomers. Almost 80% of these monomers are easily dissociable in high salt concentration and do not participate in protein synthesis (free ribosomes). Free ribosomes are not an artifact due to reassociation of subunits obtained during separation because labeled 40S particles added to the cytosol before separation were not incorporated into the free ribosomes. A number of pulse-chase experiments during the past year established that free ribosomes derived from mono- and polysomes as they aged. It appears that a new formed ribosome is utilized 10-15 times in protein synthesis and is then inactivated to the free ribosome form. The functional difference between free ribosome and active monosome is also expressed by the sedimentation behavior. 40S subunits derived from monosomes sediment slightly more slowly than 40S subunits derived from free ribosomes. Whether this difference in sedimentation indicates a lack of some component which is indispensable for dissociation of the ribosome and, therefore, excludes it from participating in protein synthesis remains an open question. When lymphocytes were stimulated to grow, free ribosomes could dissociate and enter protein synthesis. In fact, for the first 20 hrs of growth, new ribosome synthesis was

not necessary since the pool of free ribosomes could compensate for it. The activation of free ribosomes did not require new synthesis of proteins but of mRNA as established by the use of inhibitors. Since there is no shortage of mRNA in the resting cell, the observation calls for an analysis of the kind of RNA actually required. A more extensive analysis of the functional significance of the RNA formed during lymphocyte activation is planned using interferon production by mRNA of the stimulated lymphocyte in an in vitro translating system as a probe.

Dr. Berger has analyzed the formation of heterogeneous nuclear RNA, (hnRNA) and the adenylation process which maintains the cytoplasmic pool of poly(A) bearing mRNA. She established that large hnRNA molecules are poorly represented in resting lymphocytes. Polyadenylation confers stability to the hnRNA and Dr. Berger found that in resting lymphocytes hnRNA + poly(A)RNA molecules decayed with a half life of 7 hrs. The processing of hnRNA + poly(A)RNA to poly(A)-bearing RNA should be rapid because no pool of intermediate size molecules was found. About 75% of newly synthesized hnRNA + poly(A) was found in the nucleus when no transport to the cytoplasm could be observed. Probably a fraction of hnRN + poly(A) is rapidly processed and transported to the cytoplasm for immediate use while another portion is recycled within the nucleus.

In the resting lymphocyte, the half life of poly(A)-bearing RNA was determined by pulse-chase experiments. About 80% of the newly synthesized molecules had a half life of 17 min while the remaining class did not appear to be degraded during 24 hrs of observation. The size distribution was broader for newly synthesized mRNA which suggests that mRNA is recycled within the cytoplasm. When the resting lymphocyte was stimulated to active growth, only mRNA molecules with a half life of 5-6 hrs were found, suggesting that mRNA processing may differ between growing and physiologically resting lymphocytes.

4) Role of Extranuclear DNA and Nucleotide Metabolism in Normal and Neoplastic Tissues

Dr. Schneider attempted isolation and identification of deoxyribose-containing compounds in luteal cells, obtained in the ovary by stimulation with gonadotropin and prolactin. Grains of ^{3}H-thymidine were formed associated with the endoplasmic reticulum, suggesting that DNA was, indeed present in the endoplasmic retiuclum. However, the DNA contributed by nonluteal cells impeded meaningful fractionation for biochemical studies.

The search for differences between nuclear and cytoplasmic DNA revealed that:

(a) DNA was present in melanin granules of S-91 melanoma. This DNA, however, sedimented very slowly and was quite heterogeneous in size, thus negating support for the hypothesis that melanine granules were mitochondrial mutants.

(b) 85% of mitochondrial DNA renatured very rapidly ($COT_{\frac{1}{2}} = 0.025$) as a single component; about 65% microsomal DNA renatured more slowly ($COT_{\frac{1}{2}} = 0.16$)

but still as a single component; however nuclear DNA renatured slowly, over a broad range of C_oT values and with no well-defined components. In contrast to nuclear DNA, microsomal and mitochrondrial DNA can be considered distinctive types, rich in repeated sequences.

(c) Methylcytosine was found in nuclear, mitochondrial, and microsomal DNA samples of rodent liver and its presence or quantity could not serve as a basis for differentiating DNA types obtained from the same tissue.

(d) A method for isolating zymogen granules from mitochrondria was developed and their DNA content is under investigation.

Drs. Anderson and Liacouras continued their work on pyrimidine metabolism, concentrating on the role of UMP. Uridine-cytidine kinase was isolated and purified 1800-fold. Using the purified enzymes, they showed that: (1) Only uridine and cytidine and their analogs serve as phosphate acceptors but the enzyme has a broader specificity for phosphate donor substrate. (2) The initial velocity pattern for the phosphorylaton of either uridine or cytidine suggests that the reaction proceeds by sequential addition of ATP and nucleoside to the enzyme followed by sequential release of the nucleoside monophosphate and ADP. (3) K_m of enzyme was 1.5×10^{-3} for ATP with either uridine or cytidine. Vmax = 1.83 μmole for cytidine kinase. (3) Studies on the competition between uridine and cytidine suggest that phosphorylaton is catalyzed by a single enzyme protein at a single site rather than by isozymes at two separate sites.

A correlation between biochemical characteristics of nucleoprotein and the initiation of the neoplastic event is being sought by Dr. Rotherham. Results are not yet available.

5) The Physiological and Behavioral Control of Food and Water Intake and its Relation to Cancer Cachexia.

Dr. Morrison is presently working alone on this difficult problem. He has completed a new data acquisition system for studying patterns of heat production in relation to feeding behavior and influence of the tumor thereon. Extensive analysis of the normal control of feeding has demonstrated that this is a highly redundant system; however, as the tumor develops, a successive deletion of different components of the feeding control mechanism occurs until a catastrophic collapse ensues with cachectic hypophagia. The elements which influence food intake, i.e., feeding activity, efficiency of feeding, etc., are being analyzed with the objective of developing ways of understanding the basis for behavioral adaptation to feeding patterns and breaking thereof.

A project which I hope to integrate into the nutrition program, is the preparation of amino acid analogs obtained by acylation. Dr. Otani has prepared 25 chloroacetyl and 10 propionyl derivatives of amino acids and has tested their growth inhibitory activity on a Lactobacillus casei system.

6) The Effect of Ionizing Radiations on Tissue Components and the Intact Whole Host.

Two aspects of this problem are being investigated: the effects of ionizing and UV radiation on nucleic acids and proteins and the consequences on the host of tissue destruction by radiation.

Dr. Riesz is studying the effects of ionizing radiations on deaerated aqueous solutions of cytosine, thymine, uracil, adenine, guanine, and the corresponding nucleosides and nucleotides. From electron spin resonance spectra, he established that H or OH radicals, produced by γ irradiation of water, substitute specific positions: for cytosine, the 5 position of the 5-6 double bond; for 2'-deoxycytidine and 2'-deoxycytidine-5'-monophosphate the 6 position; for 2-deoxyribose, the 2' or 5' position; for thymine, the C5, N1 or N3, and C6 positions; and for uracil, C5. By trapping free-formed radicals with 2 nitroso-2-methylpropane, stable nitrosyl radicals were formed at the N1 and C6 positions of uracil. Reactions of hydrated electrons with phenylalanine, histidine, and serine produced radicals of the type $CH(COO-)CH_2R$; with lysine, tryptophan and tyrisine = CH type; with glycyl glycylglycine and DL-alanyl-DL alanine, α carbon radicals were formed. The effect of light of various wave lengths on γ-irradiated "wet" DNA is also being studied. The decay of pyrimidine and purine radical ions and a transformation of the thymine anion radical are being followed.

Toxic levels of irradiation are used by Dr. Uphoff to destroy the marrow of inbred strains of mice and to study bone marrow transplantation. The general objective is to understand the biological laws governing the reaction which occurs when hybrid tissue is transplanted to a parental strain. Parent-anti-hybrid and hybrid-anti-parent reactions range from subclinical to lethal events. One exception was found: parent-anti-hybrid reactions were minimal when the maternal strain of this hybrid cross was syngenic with the parental strain marrow. However, this parental strain marrow produced a parent-anti-hybrid reaction in the reciprocal hybrid where the maternal strain of the hybrid was allogenic with the hybrid donor. Maternal influences were a determinant factor in creating marrow graft adaptation. To study this event, ova-transfer substrains and the strain of the ancestral foster mother were compared. When ova-transfer substrain was derived from the female of the hybrid cross, the production of hybrids was uneventful. However, when the ova-transfer substrain derived from the ancestral foster mother, the young hybrids became anemic and runted. Runting disappeared when the mother's milk was eliminated. The second litter usually did not runt. Thymic tissue deficiency appears to be a major element of the syndrome.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00306-13 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Modification of amino acids in search of possible anti-tumor agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Theodore T. Otani Research Chemist LPP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Nucleic Acids Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

To prepare new amino acids and amino acid analogs and their derivatives by organic chemical methods in search of compounds that destroy cancer cells.

PHS-6040 (12-75)

Project Description:

Objectives: To prepare chemical compounds which preferentially destroy cancer cells by mechanisms other than those of agents presently being used in cancer chemotherapy. Our studies with the amino acid analog, β-hydroxy-norleucine, prepared in this Laboratory, have shown that acylation of certain amino acid analogs "activated" these compounds to inhibit the growth of certain microorganism where no such activity was observable with the free amino acid.

The immediate objectives of the project have been:

(a) To prepare various acyl derivatives of other amino acids and amino acid analogs and to note if these compounds exhibit similar microbial growth-inhibitory capacities.

(b) To prepare other new amino acids analogs, which by their structural resemblance to essential natural amino acids, may be potential anti-metabolic and, hence, growth-inhibitory agents.

(c) To prepare derivatives of these amino acids to contain organic functional groups which may potentiate the growth-inhibitory as well as increase the cell permeability capacities of the molecules.

(d) To test these new compounds, for anti-tumor activity, first in microbial anti-tumor screening system, and if found active, and in mammalian systems when obtained in sufficient quantities.

Methods Employed: The β-hydroxyamino acids were prepared by methods developed in this Laboratory. An appropriate aldehyde is condensed with glycine in the presence of catalytic amounts of cupric ions (or with glycine copper complex) in a basic medium under conditions which are empirically determined. Isolation of diastereoisomers was accomplished by partition column chromatorgraphy. Resolution of optical isomers was accomplished by asymmetric enzymatic hydrolysis using hog renal acylase and bovine carboxy-peptidase. Acylation was accomplished by the Shotten-Baumann reaction. Microbiological assays were carried out as described for riboflavin assay, but with minimal quantities of the vitamin added to give maximum growth.

Major Findings:

(1) Twenty five chloroacetyl and ten propionyl derivatives of amino acids and amino acid analogs have been prepared. These were characterized by elemental analysis, optical rotation, Van Slyke nitrous acid determination of free amino groups, and melting point determinations.

(2) These compounds, with their un-acylated mother compounds, (many of which were synthesised and characterized in this Laboratory), were tested for growth-inhibitory activity in a Lactobacillus casei system. While the propionyl derivatives showed no remarkable activity, the chloroacetyl derivatives exhibited modest but significant activity.

(3) In general, where activity was noted, the L-isomer exhibited somewhat greater inhibition than the corresponding D-isomer. However, in some cases, the D-isomer also showed some inhibition.

(4) Where appreciable inhibition was observed in the free amino acid, as in p-fluorophenylalanine, the acylated derivative showed no activity over that shown by the free amino acid. Indeed, such derivatives exhibited less activity than that shown by the parent amino acid.

(5) When two "active" acyl derivatives were combined and assayed, the inhibition observed was not additive.

(6) The extent of inhibition in the microbial screen did not appear to be related to the hydrolytic rate of the acyl amino acids, since susceptability of the acyl compounds to acylase and carboxypeptidase did not parallel the degree of inhibition.

(7) Of the acyl amino acids tested, chloracetyl-D-β-hydroxynorleucine-B was the most active.

Significance for Cancer Research and the Program of the Institute: That amino acid metabolism may be a susceptible area of action in cancer therapy was demonstrated by the efficacy of asparaginase in certain tumors which required asparagine. Amino acid deprivation, either by the removal of the amino acid or by the substitution with an analog may be effective in inhibiting tumor growth.

The unnatural amino acids synthesized under this program are close structural analogs of essential natural amino acids, differing in some cases only in the presence of a single hydroxyl group adjacent to the α-amino group. Hence, β-hydroxynorleucine differs from norleucine only in the presence of a hydroxyl group in the β-position of the carbon chain of the amino acid. β-Hydroxynorleucine is a structural analog of methionine, differing from the latter in having a methylene group in place of the sulfur atom in the γ-position of the chain. Indeed, β-hydroxynorleucine, even as a mixture of four isomers, exhibited inhibitory action in two microbial systems. β-Hydroxymethoxinine and β-hydroxymethionine are even closer analogs of methionine than is β-hydroxyhomomethionine, the former differing from methionine in possessing an oxygen atom in place of the sulfer atom and in possessing a β-hydroxyl group, and the latter only possessing a β-hydroxyl group.

The chain-halogenated amino acids, similarly, are close structural analogs of essential amino acids. For example, γ-dichlorothreonine would be an analog of leucine, since the size of the chlorine atom is approximately that of the methyl group; also γ-trifluorothreonine would be a close analog of threonine since the atomic radius of the fluorine atom is essentially that of hydrogen atom.

The enhancement of activity of the amino acid, and amino acid analogs upon acylation appears to be of importance in the cancer program in view of the fact that one serious drawback in cancer chemotherapy has been that of the inaccessibility of the cytotoxic agent to the cell. It is possible that alteration of the molecular charge by acylation, and the introduction of a cytotoxic group, in the form of the chloroacetyl radical, has provided a molecule that is more permeable to the cell and at the same time, once within the cell, capable of releasing the cytotoxic agents by the action of intracellular acylases. Objective 6, Approach 2.

Proposed Course of Research:

(1) To continue comparative studies of the effect on the growth-inhibiting activity of other acyl groups attached to amino acids and amino acid analogs studies in this series.

(2) To prepare γ-halogenated β-hydroxyamino acids, which are possible antimetabolites of threonine and leucine.

(3) To prepare N-trifluoroacetyl derivatives of some of the analogs and compare the activity of these compounds with that of the corresponding acetyl, chloroacetyl and propionyl derivatives.

(4) To prepare analogs of tryptophan, i.e. β-hydroxytryptophan, starting with indole-3-carboxyaldehyde and glycine-copper complex.

(5) To test the compounds which have been found to be active in the microbial screen, in mammalian tumor systems.

Publications:

Otani, T. T. and Briley, M. R.: β-Hydroxynorleucine: Separation of its isomers and biological studies. J. Pharm. Sci. March, 1976. In press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00380-20 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Deoxyribose containing compounds in normal and cancer tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. C. Schneider Head, Nucleic Acids Section LPP NCI

COOPERATING UNITS (if any)

Drs. E. Shelton, E. L. Kuff, and R. Gantt, LB, NCI; Dr. G. H. Smith, LMBGY, NCI; Dr. W. Kidwell, LPP, NCI

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Nucleic Acids Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objectives of this project are to isolate and identify deoxyribose-containing compounds other than DNA from normal and tumor tissues; to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal, precancerous, and cancer tissues; to isolate DNA from subcellular structures other than nuclei; to study the physical and biochemical properties of extranuclear DNA; to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

PHS-6040 (12-75)

Project Description:

Objectives: Since the replication of DNA is an obligatory prerequisite for cell division, the study of DNA synthesis and its control occupies a position of unique importance in cancer research because the malignant cancer cell displays continuous and uncontrolled division. The objectives of this project are to isolate and identify deoxyribose-containing compounds other than DNA from normal, precancerous, and tumor tissues, to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal, precancerous, and cancer tissues; to isolate DNA from subcellular structures other than nuclei, and to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

These studies are aimed at uncovering differences between normal and malignant cells that can be exploited to prevent the formation of cancer cells or to promote the destruction of cancer cells already generated. (These studies support most closely Objective 3: To prevent conversion of cells to those capable of forming cancer and Approach 1: Nature and modification of the precancerous state, of the Nactional Cancer Plan).

Methods Employed: Nuclei, mitochondria, zymogen granules, and microsomes were isolated from rat or mouse tissues by differential and/or density gradient centrifugation as well as by centrifugation in the zonal rotor. Nucleic acids were extracted from tissue fractions using sodium lauryl sulfate (SLS) and phenol. DNA was purified from these extracts by treatment with ribonuclease and passage through Biogel P-60, by banding in CsCl, by chromatography on hydroxylapatite, or by treatment with NaOH to remove RNA and small molecules.

DNA samples were examined by means of analytical CsCl density gradient centrifugation, melting behavior, and amount of labeled thymidine incorporated. DNA was measured by a modification of the diphenylamine reaction. Enzyme markers were used for estimating purity of isolated subcellular fractions.

The reassociation kinetics of DNA were measured on hydroxylapatite columns using phosphate buffers of prescribed concentrations for elution of denatured and reassociated DNA (Britten, Methods in Enzymology). The DNA samples used in these studies were either sheared in a high speed blender or treated with acid and alkali to obtain smaller fragments (Kidwell, unpublished).

For the study of methyl cytosine contents, the DNA samples were hydrolyzed to the free bases in formic acid. The bases were then chromatographed in a high pressure liquid chromatography system and quantitated with a two wave length ultraviolet monitor.

Major Findings: During the past year a number of goals were realized relative to determining whether DNA was present in cytoplasmic organelles other than mitochondria. Several of these have done much to strengthen our previous findings on the association of DNA with microsomes.

DNA in luteal cells (with Drs. Smith and Kidwell). The ovary, stimulated with gonadotrophin and prolactin, was selected as a possible model system for studying the function of DNA in the endoplasmic reticulum since it had been shown by Enders that the endoplasmic reticulum underwent tremendous proliferation in the luteal cells in response to the hormonal stimulus. The hope that this system could be used for biochemical studies was not realized, however, because the effects observed in the luteal cells were obscured by the DNA contributed by the many other cells present in the ovary. However, radioautographic studies of the luteal cells showed that many grains from ^{3}H-thymidine were present in the cytoplasm and a number of these were associated with the endoplasmic reticulum. This was an important finding since it gave the first direct evidence for the presence of DNA in the endoplasmic reticulum. This work has now been published in Experimental Cell Research.

DNA in melanin granules (with Drs. Shelton and Kuff). In another paper published in the Journal of the National Cancer Institute, we reported our findings on the association of DNA with melanin granules isolated from the S-91 melanoma and Amphiuma liver. This work was undertaken because it had been hypothesized by Woods and Dubuy that melanin granules were mitochondrial mutants. Our results do not affirm this theory because the DNA associated with melanin granules did not resemble mitochondrial DNA. The melanin DNA turned out to be a rather peculiar DNA since it sedimented very slowly and appeared to be quite heterogeneous in size.

Reassociation kinetics of liver DNA. Our previous studies of nuclear, mitochondrial and microsomal DNA showed that microsomal DNA could be differentiated from nuclear and mitochondrial DNA only on the basis of ^{3}H-thymidine incorporation--microsomal DNA incorporated much more isotope than nuclear DNA but much less than mitochondrial DNA. We have now studied the renaturation kinetics of these three types of DNA and found them to be strikingly different. While 85% of mitochondrial DNA renatured very rapidly ($C_0t_{\frac{1}{2}}$= 0.025) as a single component, microsomal DNA renatured more slowly ($C_0t_{\frac{1}{2}}$=0.16) but still mainly as a single component (about 65%). Nuclear DNA renatured slowly over a broad range of C_0t values (50% renaturation at C_0t=200) with no well defined components. These results indicate that in contrast to nuclear DNA microsomal and mitochondrial DNA contain many repeated sequences and that microsomal DNA like mitochondrial DNA can also be considered to be a unique and distinctive type of cytoplasmic DNA.

Methyl cytosine content of liver DNA (with Dr. Gantt). In a search for other differences between nuclear and cytoplasmic DNA samples, a comparison of the methyl cytosine contents is being made. The results so far indicate that nuclear, mitochondrial and microsomal DNA samples all

contain this base. It is not yet clear whether the quantities in the different samples are the same. On the basis of the results so far it would not appear that methyl cytosine will serve as a basis for differentiating different types of DNA obtained from the same tissue. On the other hand, the results add fuel to a controversy in the literature, since some workers claim that mitochondrial DNA contains no methyl cytosine.

Zymogen granules from the pancreas (with Dr. Smith) Zymogen granules of the pancreas were chosen for study as another example of a cytoplasmic organelle that might contain DNA. Previous studies by Greene, Hirs, and Palade showed that pancreatic zymogen granules could be obtained in pure form as judged by electron microscopy. These workers also reported that DNA was present but did not attempt to show that it was a unique DNA. In attempting to isolate these granules by methods described in the literature, we met with little success since the yields of zymogen granules as measured by amylase activity was low while contamination by mitochondria was constant. We have now developed a simple method for isolating these granules in yields of > 60% in the zonal ultracentrifuge rotor in a single run. The granules are pure as judged by electron microscopy and assays for amylase, succinic dehydrogenase, and DPNH-cytochrome c reductase activities. These results are now being written up for publication. Whether they contain DNA will be considered in future experiments.

Significance for Cancer Research and the Program of the Institute: The above findings add greatly to our fundamental knowledge about the localisation of DNA in the cell. The results indicate that although the bulk of the cellular DNA is found in the nucleus, small quantities of DNA are also present in cytoplasmic organelles. The functions of cytoplasmic DNA are being investigated. One theory is that the microsomal DNA may be a "messenger" DNA. Whatever the role these nucleic acids ultimately assume, it seems clear that cytoplasmic DNA needs to be reckoned with in experiments on carcinogenesis, which so far have focused only on nuclear DNA. The fact that they have not been studied from this standpoint is not surprising, however, since the amounts of cytoplasmic DNA are so small and there was uncertainty about whether some of them, such as microsomal DNA, were truly cytoplasmic components. The uncertainty about microsomal DNA appears to be eliminated by the results described in this report and makes their investigation in tumors and in tissues during carcinogenesis of paramount importance.

Proposed Course of Research: The study of cytoplasmic DNA will be continued and the reassociation kinetics comparisons of the various liver DNA samples will be completed. With the availability of a reproducible method for isolating pure zymogen granules, the question of whether they also contain DNA can be taken up and, hopefully, quickly answered. The studies on melanin DNA should be expanded to a study of its reassociation kinetics since such studies could tell whether this DNA is unique or closely related to nuclear DNA. Studies of cytoplasmic DNA in tumors should also be begun.

Publications:

Schneider, W. C.: Enzymatic activities of subcellular fractions. In Fasman, G. D. (Ed.): Handbook of Biochemistry and Molecular Biology. Cleveland, Ohio, Chemical Rubber Co. (in press)

Schneider, W. C., Shelton, E. and Kuff, E. L.: J. Natl. Cancer Inst. 55: 665-670, 1975.

Smith, G. H., Kidwell, W. R. and Schneider, W. C.: Exptl. Cell Research 96: 321-334, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00919-13 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pattern of Cytochemical Features in Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: James C. Reid Supervisory Research Chemist LPP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pathophysiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

SUMMARY OF WORK (200 words or less - underline keywords)

New techniques coming into being for automatic cytology in flow systems require improved methods for tissue disaggregation and selective staining of peculiar cells. This project is aimed at the development of approaches to these two problems.

Project Description:

Objectives: Development of technique for chemical examination of individual cells.

Methodology: Physical and chemical methods for manipulation of macromolecules and cellular aggregates.

Major Findings: The possibility of analyzing urine for macromolecules of possible diagnostic significance has been examined. The idea was that a "chemical amplifier" might be devised which would be the basis for analytical methodology sufficiently sensitive to deal with the very small concentrations of such molecules to be expected. In particular, the amounts of replicative molecules might be augmented by application of enzymic replication procedures and appropriate selection of templates might permit a very selective augmentation of individual species. However, the literature indicates that available systems are too complex and too poorly defined to justify experimental trials.

Emphasis has been redirected now to examination of individual cells rather than urine and a relationship has been established with the cytology automation group in the Laboratory of Pathology, which is conducting both an intramural and a contract research program. Presently, the literature is being surveyed in an effort to formulate experimental approaches to two problems of special significance to this program: 1) disaggregation of cells joined by desmosomes and other surface structures and 2) development of new types of fluorescent markers more specific for peculiar cellular features than those now available. In addition, considerable time is being spent assisting the group with the analysis of research contracts.

Significance for Cancer Research and the Program of the Institute: Present activities relate to development of technique for mass screening by automatic flow systems. Objective 5, Approach 4.

Proposed Course of Project: Indeterminate, depending upon the outcome of literature studies in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00936-06 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Recovery from effects of radiation in terms of tolerance to subsequent treatments

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Willie W. Smith Research Physiologist LPP NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pathophysiology

SECTION
Radiation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	1.0	4.0

SUMMARY OF WORK (200 words or less - underline keywords)

In mice given an initial irradiation followed by subsequent challenge it appears that marrow and not gut damage is responsible for the long-recognized but poorly understood persisting loss in ability to tolerate whole-body irradiation.

Project Description:

Objectives: To identify the physiological basis of the radiation injury responsible for a persisting reduction in ability to survive subsequent irradiation, and to understand the recovery pattern in mice given radiation to the gastrointestinal tract in the absence of severe marrow damage.

Major Findings: Mice given an initial whole body exposure of 526R (lethal to less than 2%) and subsequently challenged for LD_{50} determination showed recovery equivalent to about 30% of the dose in 24 hours, 66% in 7 days and 85% in 6 weeks, results similar to those previously reported for various exposure schedules (e.g., Blair, 1952). When the mice were challenged with their legs shielded, however, recovery to the control level was complete in 7 days, thereafter showing only minor, nonsignificant variations. Thus in gastrointestinal recovery, the well-known residual effect of whole body irradiation was absent. Since the initial dose of 526R received by the gut was well below the lethal range, this was increased to 900R with the legs shielded (2% lethal) and recovery followed as before. In 24 hours this was equivalent to 40% of the initial dose, in 7 days to 80%, and in 3-4 weeks to approximately 100%. In general the two curves for mice with sublethal marrow exposure were qualitatively similar, showing no evidence of a residual irreparable damage. Therefore we infer a relationship to hematopoietic and not to gastrointestinal radiation damage.

Significance for Cancer Research and the Program of the Institute: Although clearly important in cancer research and therapy, specific alterations responsible for failure of complete recovery in mice surviving whole body irradiation have not yet been demonstrated. The present experiments appear to eliminate gastrointestinal damage as the basis of this component of radiation injury.

Proposed Course of Project: We hope to complete the present project and to resume some earlier incomplete studies of post-irradiation tolerance to stathmokinetic agents.

Summary of Proposed Work: In certain cases we have found it possible to give radiation and vinblastine on a schedule that produces greater damage to the tumor than to the host. If time allows I hope to complete these studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00941-19 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunogenetic factors affecting bone marrow transplantation in irradiated mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Delta Uphoff Research Biologist LPP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

Bone marrow transplantation in lethally irradiated mice was used to investiage immunogenetic factors affecting the severity of the graft-versus-host reaction. Immunologically competent cells derived from marrow grafts are able to distinguish qualitative and quantitative differences in expression of tissue antigens. An unresponsive state requires an equilbrum between the ability to respond and the antigenic expression of tissue antigens. Maternal influences produce phenotypic modifications in both the ability to respond and the expression of tissue antigenicity. This naturally occuring adaptive mechanism may be experimentally manipulate a) to improve survival by reducing the severity of the graft-versus-host reaction for purposes of a successful marrow graft or b) to specifically increase the immune response to produce a desired rejection as in the case of immunotherapy of tumors.

Project Description:

Objectives: To facilitate bone marrow transplantation as an adjunct to administrating toxic levels of irradiation or chemotherapeutic agents used in treating cancer. Bone marrow transplantation is also useful for treatment of combined immume deficiency diseases, aplastic anemia and accidental over exposure to irradiation and toxic chemicals.

Methods Employed: Multiple inbred strains and substrains of mice representing the major histocompatibility (H-2) genotypes, reciprocal F_1 hybrids homozygous or heterozygous for their H-2 alleles, and especially derived substrains are exposed to lethal total-body X irradiation and engrafted with marrow cells from different donors to test effects of genetic disparity, sex and parity status on engraftment and severity of graft-versus-host (GVH) reactions and their prevention.

Major Findings: Immunologically competent cells derived from marrow grafts recognize and respond according to qualitative and quantitative differences in expression of tissue antigens of recipients and the responsiveness of the donor strain. When marrow grafts are of hybrid tissue a hybrid-anti-parent reaction may occur because parental strain recipients are quantitatively more antigenic than the grafted tissue. Genetic conditions under which these graft-versus-host reactions occurred were investigated by using 4 pairs of reciprocal hybrids representing 8 different parental strains. The genetic disparity differed for each hybrid. Comparisons were made between males and females, and between hybrid-anti-parent and parent-anti-hybrid reactions. These comparisons revealed that the severity of GVH reactions were a function of relative antigenicity and responsiveness of parental strains. Both parent-anti-hybrid and hybrid-anti-parent reactions range from no apparent reaction to severe lethal ones. Parent-anti-hybrid reactions were as severe or more severe than hybrid-anti-parent reactions with one exception. Under genetic conditions where there was a difference in H-2 genotype but no appreciable parent-anti-hybrid reaction (which may indicate reduced responsiveness) there was a significantly more severe hybrid-anti-parent reaction. Under these experimental conditions this parental strain was not particularly responsive to one hybrid yet it was a strong antigenic stimulus for immunologically competent cells of this hybrid graft. Parent-anti-hybrid reactions were minimal when the maternal strain of this hybrid cross was syngeneic with the parental strain marrow. However, this parental strain marrow produced a parent-anti-hybrid reaction in the reciprocal hybrid where the maternal strain of the hybrid was allogeneic with the marrow donor indicating that this strain was responsive to appropriate antigenic stimuli, i.e., there was an additive effect of a quantitative and qualitative increase in antigenicity. Differences between the reciprocal hybrids result from maternal modifications in expression of paternally derived tissue antigens. This maternal influence created conditions suitable for adaption of marrow grafts that result in unresponsive states. Marrow stem cells that establish the allogeneic

hematopoietic systems of these chimeras are more sensitive to subtle differences in antigenicity than differentiated cells used for in vitro test systems for selection of marrow donors. Although improving tissue typing methods is beyond the scope of this project, a typing method that is sensitive enough to detect qualitative difference in expression of H-2 specificaties in reciprocal hybrid mice should produce better donor selection than is presently available.

Maternal influences on ova-transfer substrains were investigated by comparing results of experiments involving strains of origin and their derived substrains. The new aspect of this investigation involved production of reciprocal hybrids between the ova-transfer substrain and the strain of the ancestral foster mother. Data obtain with these hybrid were needed for comparison with those for hybrids between the strain of origin and that of the ancestral foster mother used for the derivation of the substrain. When the ova-transfer substrain represented the female of the hybrid cross the production of hybrids was uneventful. However when the female represented the strain of the ancestral foster mother, the young hybrids became anemic, runted and frequently died during the third week of life. This runt disease occurred in only one direction. A similar runt disease was described by Joy Palm in one hybrid cross between inbred rats. However in mice, of 3 ova-transfer substrains tested, all exhibit this phenomenon. This maternal-anti-hybrid reaction appears to result from transfer, via milk, of maternal lymphocytes primed by the first pregnancy. A second pregnancy apparently resulted in the formation of blocking antibodies or some other adaptation since runt disease did not usually occur in second litters. This hypothesis is consistant with previous experience that marrow from maternal donors produced a more severe GVH reaction after one litter than that produced by virgin donors and a less severe reaction after 3 litters, i.e., one litter sensitizes while multiple litters result in unresponsiveness. In mice there is no evidence that maternal cells form a functional transplant in the hybrid fetuses as was postulated for rats since mice are normal at birth and after runting may recover when deprived of their mothers milk. The virtual absence of thymic tissue sets these deaths apart from deaths from other causes. The possible relationship between this syndrome and athymic conditions in other mammals has not yet been established. Attempts to determine the nature of modifications that produce runting syndromes are underway.

Significance for Cancer Research and the Program of the Institute: GVH reactions are primary obstacles for clinical marrow transplantation. It is imperative that both production and prevention of this reaction are investigated in animal models where well known genetic characterestics are available. Since the most readily available marrow donors are usually family members studies of maternal influences on immune responses are an integral part of this investigation. Maternal-fetal interactions may increase or decrease the severity of GVH reactions under defineable

experimental condition. Although maternal influences are investigated in rodents they represent an adaptive mechanism with general principles that should apply to all mammals. There are clinical cases where typing methods indicate compatibility yet severe GVH reactions occur. Similarly marrow from maternal donors may be either compatible or produce a severe reaction under both clinical and laboratory conditions. The in vivo experience using animal models indicates that in vitro typing methods are not sufficiently sensitive to detect maternal influences. This naturally occurring adaptive mechanism is available for manipulation and in vitro methods have been successfully used to control the severity of the GVH reaction by using this mechanism in mice. However more information on the nature of this mechanism is require before predicable results may be obtain for application to other animal systems. Information obtained in these experiments is applicable to organ transplantation and for immunotherapy of tumors where similar adaptive mechanisms probably effect immune surveilance. Objective 6, Approach 1.

Proposed Course of Project: No changes in the course of this investigation are anticipated. The investigation of the hybrid-anti-parent reaction is complete enough to allow for further investigation of maternal influences. The experiments using reciprocal hybrids in which derived substrains are crossed to the strain of the foster mother are now featured. Whenever feasible these experiments will include tests of X and Y chromosome effects. Other aspects of this project reported in previous year are being continued according to the most efficient utilization of available space and mouse strains.

Publications:

Uphoff, D. E.: Comparative survival of lethally irradiated inbred male mice inoculated with marrow from virgin or multiparous female donors. J. Natl. Cancer Inst. 54: 1343-1348, 1975.

Uphoff, D. E.: Male-specific antigens: Evidence for allelic differences in reciprocal hybrid mice. Transplantation 20: 78-81, 1975.

Uphoff, D. E.: Hybrid-versus-parental strain reaction. I. Comparative mortality in allogeneic and parental strain male and female mice. J. Natl. Cancer Inst. 55: 1213-1215, 1975.

Uphoff, D. E.: Maternal influences: Their immunologic aspects. In Proceedings of the Workshop on Basic Aspects of Freeze Preservation of mouse strains. Mühlbock, O. (ed) Gustav Fischer Verlag, Stultgart 1976, pp 85-102.

Publications: continued

Uphoff, D. E.: Hybrid-versus-parental strain reaction: II. Comparative mortality in parental strain mice of different H-2 genotypes. J. Natl. Cancer Inst. 56: 163-166, 1976.

Uphoff, D. E.: Hybrid-versus-parental strain reaction. III. Comparative mortality in parental strain mice of the $H\text{-}2^{k}$ genotype. J. Natl. Cancer Inst. 56: 409-411, 1976.

Uphoff, D. E.: Mechanism of graft-versus-host reaction: An immunogenetic appraisal. Transplantation Proc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00942-17 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of γ-Rays on Nucleic Acids and Proteins: Protective and Sensitizing Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Riesz	Research Chemist	LPP	NCI
OTHER:	S. Rustgi	Visiting Fellow	LPP	NCI
	A. Joshi	Visiting Fellow	LPP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the effects of ionizing and ultra-violet radiation on nucleic acids and proteins and to investigate the mechanism of action of radioprotective and radiosensitizing chemicals, since these can be potentially useful in radiation therapy.

The present report includes electron spin resonance studies of the radicals produced by γ-irradiation of aqueous solutions of nucleic acids, proteins and their constituents. The effects of visible and ultra-violet light on γ-irradiated wet DNA at 77°K were investigated.

PHS-6040
(12-75)

Project Description:

Objectives: The effects of ionizing and ultraviolet radiation on biological macromolecules and their constituents are being investigated. For reproductive death, DNA is the target molecule in viruses and is at least a part of the target molecule in bacteria and mammalian cells. Radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals or by the "indirect effect" where radical species formed in the surrounding medium by radiation react with DNA. In the case of water, these species are hydrated electrons, hydrogen atoms and hydroxyl radicals. In the case of radiation damage to the chromosome, radicals formed in the nucleohistone may cause damage to the DNA.

In the chain of events that leads to loss of biological activity, free radicals play an important role. We have found recently that the addition of certain metal ions to DNA in the ratio of 1 metal ion per 10 nucleotides, can reduce the radical yield by a factor of about 10.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity of these macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

Methods Employed: Nucleic acids, proteins and their constitutents were γ-irradiated either in the solid state at 2×10^{-5} torr or in aqueous solutions in a 500-curie Cobalt γ-source. Electron spin resonance studies were carried out with a Varian E-9 ESR Spectrometer connected to a Nicolet Lab 80 computer. For photolysis studies at specified wavelengths, a 1000-watt high pressure Mercury-Xenon arc source and monochromator were employed.

Major Findings:

1. Spin-Trapping of Radicals produced by γ-Radiolysis of Aqueous Solutions of Nucleic Acids and their Constituents. (with A. Joshi, S. Rustgi)

The effects of ionizing radiation on deaerated aqueous solutions of cytosine, thymine, uracil, adenine, guanine and of the corresponding nucleosides and nucleotides were investigated. Some polynucleotides and DNA were also studied. The hydroxyl radicals, hydrogen atoms and hydrated electrons produced by γ-irradiation of water react with the organic solutes to produce short-lived radicals. In some cases, these can be trapped by addition of 2-nitroso-2-methylpropane to form stable nitroxyl radicals. From electron spin resonance measurements of the hyperfine coupling parameters of the long-lived nitroxyl radical, the initial organic radicals produced could be identified in solutions of cytosine, thymine, uracil and their nucleosides and nucleotides.

For cytosine, two groups of ESR lines were observed which decreased at different rates at room temperature. The first group of 3 × 3 × 2 × 2 lines could be assigned to a radical formed by addition of OH or H at the five position of the 5-6 double bond. The second group of 3 × 2 lines indicated addition at the 6 position. For 2'-deoxycytidine and 2'-deoxycytidine 5'-monophosphate, the radicals due to addition of H or OH at the 6 position of cytosine were identified and an additional radical formed by abstraction of an H atom from either the 2' or 5' position of 2'-deoxyribose was also observed. For thymine, the major radical species formed in γ-irradiated deaerated solutions showed an ESR spectrum consisting of 3 × 3 × 2 × 2 lines due to addition of H or OH at the C5 position, and the minor radical species showed 3 × 3 lines without further hyperfine splitting and could be interpreted in terms of abstraction reactions at either N(1) or N(3). Both thymidine and thymidine 5'-monophosphate showed a set of 3 lines without further splittings due to addition of H or OH at C6; and also a set of 3 × 3 lines which could be explained in terms of abstraction from N(3) of thymine or from C(1') of deoxyribose. For uracil the initial radical addition of H or OH occurred at the C5 position. Two spin-adduct radicals were formed by addition to the nitroso spin trap at N1 and, to a lesser extent, at C6. After γ-radiolysis of concentrated gels of DNA, the ESR spectra of the spin-adducts consisted mainly of broad lines which could not be further resolved. In addition, two sets of sharp lines were observed which decayed rapidly. The slower set decayed with first order kinetics. These sharp lines are due to low molecular weight radiolysis products and their definite assignment, if possible, would be of considerable interest.

Further details of the radical structures will be examined by deuteration, alkyl substitution and by a comparison of the spin-adduct yield from air-free, nitrous oxide saturated and formate ion-containing solutions.

2. Spin-Trapping of radicals produced by γ-radiolysis of aqueous solutions of amino acids and peptides. (with F. Friedberg, Dept. of Biochemistry, Howard University Medical School)

The effects of ionizing radiation on deaerated neutral aqueous solutions of various amino acids and peptides were studied, using the same methods as for DNA constituents.

For phenylalanine, histidine and serine, the ESR spectra showed sets of 3 × 3 × 2 lines which were clearly due to radicals formed by the addition of radicals of the type $\dot{C}H(CO\bar{O})CH_2R$ to 2-nitroso-2-methylpropane. These radicals were produced by reactions of hydrated electrons with amino acids which lead to scission of the $-NH_3$ group. From lysine, tryptophane and tyrosine, spectra containing 3 × 2 lines were obtained which indicate a $>\dot{C}H$ type of radical. From glycylglycylglycine and DL-alanyl-DL-alanine, sets of 3 × 4 and of 3 × 3 × 4 × 2 lines, respectively, were found which were due to α carbon radicals formed from the peptides by the abstraction reactions of hydroxyl radicals or hydrogen atoms. Preliminary experiments with polylysine, ribonuclease and collagen have given spectra with broad lines which could not be further resolved.

3. Effects of Light on γ-Irradiated "Wet" DNA at 77° K (with S. Rustgi and A. Joshi)

The effect of light on "wet" DNA containing approximately an equal weight of water and previously γ-irradiated at 77°K in the dark in vacuo was investigated. Extensive illumination with 315 ± 6, 436 ± 6 and 585 ± 6 nanometer light removed approximately 80% of the pyrimidine and purine radical ions. The radical decay could be analysed in terms of two first order processes, the "fast" decay being about 15 times faster than the "slow" decay at all three wave-lengths.

In addition to radical decay, prolonged illumination at 77° K induces a transformation of the thymine anion radical, formed by γ-radiolysis at 77° K, to the 5,6-dihydro-5-thymyl radical, which can be identified by the "octet" signal. It was found that the fraction of "octet" signal in the final ESR signal after intensive illumination is strongly wave-length dependent; the largest fraction was observed at 315 ± 6 nanometers.

Significance for Cancer Research and the Program of the Institute: Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy, (2) carcinogenesis, (3) stability of the genetic pool, (4) the suppression of the immune mechanism, and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained. Objective 6, Approach 1.

Proposed Course of Project: To continue studies on the effects of ionizing radiation on macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents will be investigated.

Publications:

Kominami, S., Wee, V. T., and Riesz, P.: Effects of metal ions on the formation of radicals and strand breaks and on hydrogen transfer in γ-irradiated DNA. Radiat. Res. 62: 422-437, 1975.

Kominami, S., Riesz, P., Akiyama, T., and Silverton, J. V: X-Ray Diffraction and Electron Spin Resonance Studies of Single Crystals of Copper (II) Doped L-Cystine Dihydrochloride Dihydrate. J. Phys. Chem. 80: 203-210, 1976.

Kominami, S. and Riesz, P.: ESR Studies of γ-irradiated wet DNA. Radiat. Res. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00944(a)-14 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Total Metabolism of Cancer Cachexia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. D. Morrison	Research Physiologist	LPP	NCI
OTHER:	Aida Shehata	Visiting Fellow	LPP	NCI

COOPERATING UNITS (if any)

Laboratory of Theoretical Biology NCI
Processing Design Section, CSL DCRT

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Energy Metabolism Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed towards identifying the causes of the nutritional depletion and general deterioration of the host, known as cancer cachexia, with a view to blocking or reversing these systemic effects of cancer so that the cancer patient could become more accessible to anti-cancer therapies. The approach to this is by investigation of total energy, water and other material exchanges, and of the physiological and behavioral control of food and water intake in normal and cancerous organisms.

PHS-6040
(12-75)

Project Description:

Objectives:

(a) Investigation of the patterns of heat production of rats with concomitant recording of feeding behavior and other activity with a view to finding how the energy expenditure, feeding and general behavior pattern are related to tumor growth, to finding the nature of the loads imposed by a tumor on its host, and to identifying the causes of cancer cachexia.

(b) Investigation of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth.

(c) Examination of possible experimental methods of simulating in non-tumor-bearing rats the cachexia and decline of food intake induced by tumor growth.

(d) Identification of functional sites and causes of breakdown of control of food intake during tumor growth.

(e) Development of conceptual models of control of food and water intake and regulation of energy and water exchange and their interrelationships for normal animals and for the cancer cachectic process.

(f) Development of quantitative and reproducible assessment of behavioral output, to yield more sensitive and earlier detection of systemic effects of tumors.

(g) Advisory and administrative activities to enable effective development and prosecution of the Diet, Nutrition and Cancer Program.

Methods Employed: The methods of indirect, total, long-term calorimetry, operant conditional responses, placement of electrolytic lesions in or stimulation of the central nervous system, continuous or programmed infusions into unrestrained animals, and methods of orthodox nutritional studies. Ballistic methods of recording and partitioning total motor activity. Computer methods of numerical analysis of serial records of tumor growth, continuous records of gaseous exchange and of change in feeding and drinking patterns in relation to total energy exchange and tumor growth. Sprague-Dawley rats and Buffalo rats are used in all animal experiments. Most tumor-bearing rats are inoculated with Walker 256 carcinoma, but other transplantable tumors are also used.

Major Findings:

(a and b) In this area the year has been devoted to developing, assembling and testing a new data acquisition system interfaced to a Wang 2200C programmable calculator, with the collaboration and assistance of Mr. J. I. Powell of the Processing Design Section, CSL, DCRT. This instrumentation, which will be applied to all areas of the work of this section, is now almost complete.

(c and d) Normal control of feeding is a complex and highly redundant system. Individual components of the system can be deleted or can be innately absent without grossly interfering with overall control (Morrison, S. D., Am. J. Physiol. 224: 1305, 1973; Cancer Res. 35: 3339, 1975). Feeding responses to different stimuli (e.g. change in nutrient density, change in environmental temperature, exogenous insulin, taste adulteration, etc.) represent different overlapping components of the control system. Previous work (Morrison, S. D., Cancer Res. 32: 968, 1972; Cancer Res. 33: 526, 1973) showed that response to nutrient density is immediately and progressively impaired by tumor growth. Responses to environmental temperature change and to exogenous insulin are also distorted and/or attenuated at subsequent stages of tumor growth: studies on these responses are in advanced progress but are still incomplete: preliminary aspects of this work have been reported (Morrison, S. D., Cancer Res. 35: 3339, 1975). Studies have been started on the response to 2-deoxy glucose (a competitive glucose analogue). It seems likely that cachectic hypophagia develops as a consequence of successive deletion of different components of feeding control until the redundancy of the normal control complex is lost and catastrophic collapse of control and of feeding ensues.

(e) Work has been started on the development of conceptual models of control of food intake that would correct errors and misconceptions of existing models (Morrison, S. D., Nature, 255: 169, 1975) and that would allow analysis and inclusion of observed changes in feeding during tumor growth. Computer analysis of long-period records of meal patterns and of energy expenditure is being developed in collaboration with Dr. F. K. Millar (Laboratory of Theoretical Biology, DCBD).

(f) The possible modes of change in feeding that can produce change in food intake (Morrison, S. D. and Coffey, N. F., J. Appl. Physiol. 34: 268, 1973) are being examined in relation to cachectic hypophagia. It has been shown that, for most of tumor growth, a decline in one mode (change in amount of feeding activity) is fully compensated by increase in another mode (change in efficiency of feeding) with no resultant change in food intake at this stage (Morrison, S. D., Cancer Res. 36: 228, 1976). This indicates that the cachectic process starts very early in tumor growth but is compensated by behavioral adaptation: only when the capacity for behavioral adaptation is exhausted do cachexia and hypophagia become overt.

(g) There has been intensive activity, in the form of advisory papers and memoranda, to a variety of Workshops and to the Advisory Committee in connection with the Diet, Nutrition and Cancer Program and in connection with membership of the Coordinating Committee of the Diet, Nutrition and Cancer Program.

Significance for Cancer Research and the Program of the Institute: The findings on control of feeding in tumor growth are throwing light on the nature of cancer cachexia and should be utilizable in the development of effective methods for improving the nutritional condition of cancer patients. The section of the National Cancer Plan that the work most closely approximates is: Objective 6 (Develop the means to cure cancers and to retard the progress of cancers not cured). Approach 4 (Enhance the host's ability to eliminate or prevent further development of cancer). It is also immediately relevant to the 1974 Amendment to the Cancer Act (Collect ..... information respecting nutrition programs for cancer patients and the relationship between nutrition and cancer).

Proposed Course of Project: Work on energy and water exchange of tumor bearers will be continued along with study on the interaction of metabolic and behavioral responses of normal and cancerous animals, with particular reference to the reasons for the changes in food and water intake and depletion of host tissues that occur during tumor growth. Objective 6, Approach 4.

Publications:

Morrison, S. D. Feeding analogues. Nature, 255: 169, 1975.

Morrison, S. D. Origins of nutritional imbalance in cancer. Cancer Res. 35: 3339-3342, 1975.

Morrison, S. D. Generation and compensation of the cancer cachectic process by spontaneous modification of feeding behavior. Cancer Res. 36: 228-233, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05205-09 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on biochemical mechanisms for control of nucleic acid synthesis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Elizabeth P. Anderson Research Biochemist LPP, DCBD, NCI

OTHERS: Alec S. Liacouras Research Biochemist LPP, DCBD, NCI

COOPERATING UNITS (if any)

Bruce A. Chabner, LCHPH, DCT, NCI

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

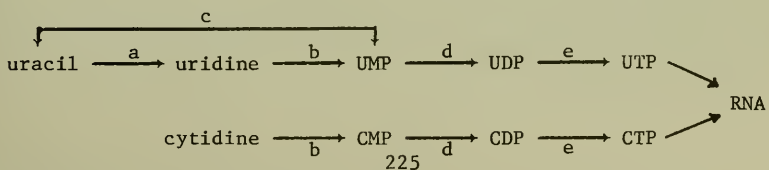
SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range objective of this project is to investigate nucleotide metabolism in normal and cancer cells. The aim is to elucidate pathways of biosynthesis, mechanisms of the enzymatic reactions involved, mechanisms of control over these reactions and pathways, and biochemical mechanisms of sensitivity and resistance to purine and pyrimidine analogs. The end-products of nucleotide biosynthesis are the triphosphate precursors of nucleic acids, and regulation of the enzymes involved in these biosynthetic steps is presumably a major factor in determining the rate of cellular nucleic acid synthesis. In view of the central role of UMP in pyrimidine metabolism, present work is focussed on reactions b, c, and d in the following pathway of pyrimidine nucleotide biosynthesis:



Studies on uridine-cytidine kinase. In mammalian tissues uridine-cytidine kinase (reaction b above) is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction has been shown to be rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end-products (Anderson et al., Biochem. Biophys. Acta 91: 380, 1964). We have now purified this enzyme from the mouse ascites mast cell neoplasm P815/S, in which we first demonstrated feedback regulation of the enzyme (Liacouras and Anderson, Arch. Biochem. Biophys. 168: 66, 1975). The purification procedure includes chromatography on DEAE-cellulose, ammonium sulfate fractionation and separation on hydroxyapatite. The final preparation has a specific activity of 3.02 u/mg, the most highly purified preparation of this enzyme yet isolated representing 1800-fold purification from the whole tumor tissue. Sensitivity to the feedback inhibition by the triphosphate end-products CTP and UTP is fully retained by the enzyme throughout the purification. We have characterized the purified enzyme with respect to stability, requirements of the enzymatic reaction, including divalent cation requirement, pH optimum, specificity of both the phosphate donor and the phosphate acceptor substrates, and molecular weight. Our most recent experiments indicate the molecular weight to be near 200,000. The enzyme exhibits a broad specificity for the phosphate donor substrate, but is much more specific for the phosphate acceptor, with only uridine, cytidine, and their analogs serving as substrates. Thus, 5-fluorouridine and the 5-aza- and 6-aza- analogs of uridine and cytidine are phosphorylated by the enzyme, but thymine riboside, the deoxyribonucleosides, and cytosine arabinoside are not phosphorylated. In recent explorations of other possible substrates for the kinase, 3-deazauridine was found to be phosphorylated, but pyrazomycin was not; both of these compounds have attracted recent clinical interest as anticancer agents and presumably might function as phosphorylated derivatives. Using the purified enzyme, we have also shown that the initial velocity pattern for the phosphorylation of either uridine or cytidine is one of intersecting lines rather than parallel lines, indicating that the reaction proceeds by the sequential addition of both substrates (ATP and nucleoside) to the enzyme to form a ternary complex, followed by the sequential release of the two products (nucleoside monophosphate and ADP). A ping-pong mechanism, in which the product of the first half-reaction is released prior to the addition of the second substrate to the enzyme, is ruled out by this initial velocity pattern. Analysis of the data by Cleland's Sequen computer program indicated a K_m of the enzyme for uridine of 1.5×10^{-4} M, for cytidine of 4.5×10^{-5} M, and for ATP, with either uridine or cytidine as phosphate acceptor, of 3.6×10^{-3} M or 2.1×10^{-3} M, respectively. The V_{max} was 1.83 μmoles phosphorylated/min/mg enzyme protein for the uridine kinase reaction and 0.91 μmole for the cytidine kinase reaction. These aspects of the kinetic studies and the analysis of the reaction mechanism have now been published (Liacouras et al., Arch. Biochem. Biophys. 168: 74, 1975). Dr. Liacouras has also completed his doctoral studies at George Washington University, with a thesis on the purification and characterization of the enzyme, and his Ph.D. degree was awarded in September, 1975. Other kinetic studies and product inhibition experiments are now in progress to determine whether the sequential addition of the two substrates to the enzyme is ordered (occurring in a prescribed sequence) or random. Results to date indicate that, with either

uridine or cytidine as substrate, the pattern of inhibition by the immediate product CMP is non-competitive with respect to the nucleoside substrate. The pattern of inhibition by CMP relative to the phosphate donor substrate, ATP, is currently being examined. A random sequential addition of substrates would result in competitive inhibition patterns for either product against either substrate. Hence, the non-competitive inhibition pattern produced by CMP against the nucleoside substrate suggests that the sequential addition of substrates to the enzyme may be ordered rather than random. Still other kinetic studies, now nearing completion, have explored the competition between the two alternate nucleoside substrates uridine and cytidine to delineate whether both are phosphorylated at the same catalytic site on one enzyme or whether the two phosphorylations are mediated by separate sites or isoenzymes. The theory of the method used has been described by Cha (Mol. Pharmacol. 4: 621, 1968). The method plots the combined velocity against the concentration of one substrate at increasing fixed concentrations of the alternate substrate, and a family of curves is obtained. When the velocity is plotted against the concentration of the better substrate (higher V_{max}, uridine) at different fixed concentrations of the poorer substrate (lower V_{max}, cytidine), the curves intersect at a point where the poorer substrate appears to have no effect on the reaction velocity. The data we have obtained are in complete agreement with Cha's theoretical analysis of competition between two alternate substrates reacting at a single catalytic site. The intersection point was shown to occur around 0.3 - 0.35 mM uridine. When the combined velocity is plotted against the concentration of the poorer substrate at different fixed concentrations of the better substrate, the curves do not intersect but rather converge toward the V_{max} of the combined reaction. Again the actual data obtained closely fit the theory. Thus, these results strongly indicate that the phosphorylation of both uridine and cytidine is catalyzed by a single enzyme protein and at a single catalytic site rather than by two separate sites or isoenzymes, each specific for one nucleoside. This work is currently being prepared for publication and will also be presented at the meetings of the American Association for Cancer Research in Toronto in May.

In collaborative studies, this laboratory served as a resource for Dr. Bruce Chabner and his group in their efforts to use uridine-cytidine kinase activity to monitor possible chemotherapeutic effectiveness of 5-azacytidine in treating patients with chronic granulocytic leukemia. Our kinase purified from the P815 murine mast cell neoplasm had been shown to phosphorylate 5-azacytidine (Arch. Biochem. Biophys. 168: 66, 1975), and Dr. Chabner's group therefore reasoned that they might be able to monitor the chemotherapeutic effectiveness of azacytidine by quantitating its phosphorylation in preparations from the human white blood cells. They were, however, unable to detect any kinase activity for azacytidine in their preparations and came to us for aid. A number of experiments were carried out to rule out error due to the known lability of 5-azacytidine, to difficulties in crude extracts, and to possibly unknown species or tissue differences in the phosphorylation of 5-azacytidine. Eventually the difficulties in detecting kinase activity for 5-azacytidine in the human cell preparations were traced to the assay methods being used, and an assay was evolved that could satisfactorily detect this

activity both in crude P815 preparations and in crude preparations from human white blood cells. Results with both preparations were comparable, with azacytidine 40-60% as effective a substrate as uridine. Dr. Chabner was then able to apply this method to his studies, but the level of kinase activity in the white blood cells proved to be generally too low to allow accurate monitoring of the chemotherapeutic potential of the pyrimidine analog.

2) Studies on UMP-CMP kinase and on uracil phosphoribosyltransferase. Mr. Harry Free, in addition to helping with research activities in progress, has also been engaged in a research project of his own to follow the levels of UMP-CMP kinase (reaction d above) and of uridine-cytidine kinase (reaction b) activities during cell growth. He has measured these activities in developing cultures of Tetrahymena pyriformis to determine how the enzymatic activities increase during growth. We have previously purified UMP-CMP kinase 300-fold from Tetrahymena (Ruffner and Anderson, J. Biol. Chem. 244: 5994, 1969) and defined its reaction mechanism (Garvey, Millar, and Anderson, Biochem. Biophys. Acta 302: 38, 1973); Tetrahymena is a rapidly growing protozoan that is unable to synthesize pyrimidine or purine rings de novo and hence must depend on anabolism from the preformed ring structures, as in the scheme above. Since UMP is the central compound in pyrimidine nucleotide metabolism, the anabolism of this compound into the triphosphate precursors of nucleic acids should be related to the rapid synthesis of nucleic acids during active growth. Mr. Free's initial study assayed UMP kinase in cell lysates at different stages of culture growth, and indicated that the increase in UMP kinase activity closely followed the increase in total protein and in cell count and did not precede the peak of cell growth. After growth leveled off, aging cells did not show an immediate decrease in UMP kinase activity, even though total protein did decrease. The kinase activity must therefore be functional in the maintenance of cell viability, probably in RNA synthesis, and is hence required both for the increased production of RNA and DNA during growth and for the RNA activity throughout cell life. Comparable and coincident studies on the increase of uridine-cytidine activity during the same growth period have also been carried out. Unlike the UMP kinase activity, the uridine-cytidine kinase activity was found to be very low in growing Tetrahymena pyriformis and was barely detectible throughout the growth period. Thus, this species of Tetrahymena does not appear to manufacture UMP from uridine even during rapid growth, although it anabolizes UMP vigorously. As previously suspected (J. Biol. Chem. 244: 5994, 1969), it appears that the predominant salvage pathway being utilized by this protozoa is the direct conversion of uracil to UMP by uracil phosphoribosyltransferase (reaction c). Studies to follow this biosynthetic activity in Tetrahymena pyriformis during rapid growth are currently in progress, and will presumably complete this series of studies.

Significance for Cancer Research and the Program of the Institute: UMP is the central compound in pyrimidine nucleotide biosynthesis in that it is the precursor for synthesis of other pyrimidine nucleotides of both the ribo- and deoxyribo- series. UMP synthesis by uridine kinase is called into play in

rapidly growing tissues such as tumor tissues, and this constitutes a difference between normal and cancer cells. It is important to discover how the enzymes for UMP synthesis and metabolism are regulated in both types of cells, since such regulation is crucial in maintaining a balance among the levels of the various nucleotide precursors of nucleic acids and functions in regulating the rate of cellular nucleic acid synthesis. (National Cancer Plan, Objective #3, Approach #2).

Proposed Course of Research: Goals for the research program in the near future include the following:

A. Studies on the purified uridine-cytidine kinase.

1) Completion for publication of the manuscript in preparation on the competition between the alternate substrates uridine and cytidine, which seems to be a clear demonstration of phosphorylation of both at the same catalytic site on the enzyme.

2) Extension of these alternate substrate studies to the competition between 5-fluorouridine and uridine to define whether this analog substrate competes with the normal nucleoside for the same, single catalytic site on the enzyme. We have already shown that 5-fluorouridine is a substrate for the purified kinase (Arch. Biochem. Biophys. 168: 66, 1975) and have further demonstrated that a decrease in uridine kinase is associated with and may well be responsible for resistance to this anticancer agent in a fluorouridine-resistant subline of the P815 murine neoplasm (Cancer Res. 23: 1270, 1963). Dr. Jane Glick, in our laboratory, recently undertook the purification and characterization of the uridine-cytidine kinase from the fluorouridine-resistant tumor subline and was able to show that both the sensitive-line and resistant-line enzymes could use 5-fluorouridine as substrate but that the resistant-line enzyme used it much less effectively. Although the resistant-line enzyme (which is present at very low levels and is also much more labile) has not been purified to the same extent or yield as the sensitive-line enzyme, it might be possible to extent the studies on competition between uridine and fluorouridine to the resistant-line enzyme as well.

3) Completion of the product inhibition studies to characterize fully the order of addition of the substrates to the enzyme and hence the full reaction mechanism for uridine-cytidine kinase. The studies still to be completed are the competition between the product CMP and the substrate ATP, between the product ADP and ATP, and between ADP and the nucleoside substrates uridine and cytidine. Current minor difficulties with detectible ATPase in some of the purified preparations of the enzyme could slow up the studies to some extent, but these experiments are straightforward and should yield eminently publishable results in the near future.

4) Confirmation of the preliminary result that pyrazomycin is not phosphorylated by this kinase, and quantitative definition of the kinetic parameters of the activity of the enzyme with the new analog 3-deazauridine and comparison of its substrate capacity in comparison with that of the normal nucleosides. It is always relevant to delineate the mechanisms by which

chemotherapeutic agents are anabolized to their physiologically active derivatives, especially from the standpoint of possible combination chemotherapy, and this study is therefore of considerable pharmacologic interest.

B. Studies on the appearance of the salvage pathway enzymatic activities during growth.

1) Completion of Mr. Free's studies on the time curves for the appearance, during growth of Tetrahymena pyriformis, of the enzymes involved in the biosynthesis and further anabolism of UMP. Mr. Free has completed the experiments on two of the three enzymes involved, and this study is therefore nearing completion, with fully publishable results. Longer range goals for the research program involve further exploration on the feedback inhibition of uridine-cytidine kinase by CTP and UTP. The patterns of inhibition by these triphosphate end-products need to be defined in more detail, relative to the substrates of the reaction. Our evidence to date suggests that the inhibition by CTP is competitive with ATP, but much more complex than simple, linear, competitive inhibition, and that it is probably non-competitive with the nucleoside substrate, but again apparently complex. No evidence of sigmoid kinetics or of desensitization of the enzyme to the regulation has yet been found, and possible allosteric qualities of the enzyme and its feedback regulation are therefore still open to question. The inhibition needs to be examined in relation to the physical chemistry of the enzyme protein. We have now found that the enzyme can be safely concentrated simply by ultracentrifugation, and we have already considered plans to examine it in the analytical ultracentrifuge to determine the degree of homogeneity as well as the molecular weight. These studies are aimed at the eventual goal of understanding what the feedback regulation means in terms of the structure of the enzyme molecule.

Publications:

Liacouras, A. S. and Anderson, E. P.: Uridine-Cytidine Kinase I. Purification from a Murine Neoplasm and Characterization of the Enzyme. Archives Biochem. Biophys. 168: 66-73 (1975)

Liacouras, A. S., Garvey, T. Q., III, Millar, F. K., and Anderson, E. P.: Uridine-Cytidine Kinase II. Kinetic Studies and Reaction Mechanism. Archives Biochem. Biophys. 168: 74-80 (1975)

Liacouras, A. S.: Purification of Uridine-Cytidine Kinase from a Murine Neoplasm and Characterization of the Enzyme with Regard to Properties and Reaction Mechanism. Ph.D. Thesis in Biochemistry, George Washington University, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05211-05 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Oligo ADP-ribosylation of Chromosomal Proteins and Cell Cycle Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Kidwell	Head, Cell Cycle Regulation Section	LPP, NCI
OTHERS:	Wishard S. Lorimer, III	Research Associate	LPP, NCI
	Peter R. Stone	Visiting Fellow	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 1/2	1 1/2	1

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: Previous experiments have revealed that nuclei of eukaryotic cells contain an enzyme which polymerizes the ADP-ribose moiety of NAD through a 1',2' ribose-ribose linkage to form poly(ADP-Ribose). The polymer is at least in part associated with basic chromosomal proteins. Synthesis of poly(ADP-Ribose) occurs predominantly at the S/G_2 transition point during the cell cycle and cell division does not proceed when polymer synthesis is blocked. The current objective is to characterize the nuclear proteins to which poly(ADP-Ribose) is attached and to elucidate the significance of the attachment of polymer to proteins in so far as cell division is concerned.

PHS-6040
(12-75)

Methods: Proteins were oligo ADP-ribosylated in vitro by incubation of isolated nuclei or chromatin with ^{3}H-NAD. Selective extraction of the protein-oligo(ADP-Ribose) moiety was achieved by virtue of its differential solubility in cold perchloric acid and its insolubility in cold trichloroacetic acid. Further resolution was obtained by polyacrylamide gel electrophoresis.

Major Findings: In contrast to our findings with rodent cells (mouse L-1210 and Chinese hamster CV79), the radioactively labeled oligo(ADP-Ribose) from human cell lines (Hela, HBL-100) electrophoresed not with the H1 histone but with a more slowly migrating protein band on acid-urea gels. The amount of this protein band was proportional to the amount of oligo(ADP-Ribose) synthesized. When the substrate NAD was omitted from the incubation, the band was totally absent from the gels. It was also reduced by including inhibitors of oligo(ADP-Ribose) synthesis in the assay or by incubation at a temperature that was suboptimal for the poly(ADP-Ribose) polymerase. Radioactivity associated with the new protein band was identified as oligo(ADP-Ribose) on the basis of its conversion to phosphoribosyl-AMP by venom diesterase.

The single protein band of oligoADP-ribosylated protein apparent on acid-urea gels split into two species when electrophoresed on SDS-urea gels. The molecular weight of the two species was estimated to be 27,500 and 15,700 daltons. On the basis of the molecular weight and the amount of protein recovered, it is estimated that there are about 10^{6} protein molecules present per cell (about 1/20 of the number of copies of H1 histone). From the amount of oligo-(ADP-Ribose) associated with the protein and the chain length of the oligomer, it is apparent that there are about 10-15 oligomer chains attached to each protein molecule. Consistent with this finding is the fact that about 10 peptide fragments bearing radioactively labeled oligomer are produced by proteolytic digestion of the oligoADP-ribosylated protein recovered from rat mammary tumor nuclei. We have developed a radioimmuno assay for quantitating the mass of poly (ADP-Ribose) in whole cells. Utilizing the assay, the amount of poly-(ADP-Ribose) was found to vary during the cell cycle with maximal levels present at S/G_2 when there was 2μg of polymer mass/10^{9} cells. Assuming a chain length similar to that synthesized in vitro this translates to about 10^{6} copies of oligomer/cell or about 1 oligomer chain per copy of the acceptor protein at the S/G_2 transition point.

Previously we demonstrated that cells treated with 5-methyl-nicotinamide, an inhibitor of poly(ADP-Ribose) polymerase, became arrested at or near S/G_2. We have now directly demonstrated with our immunoassay that the inhibitor produced a 50-60% reduction of oligo(ADP-Ribose) synthesis at this time of the cell cycle. This observation is further evidence that growth arrest is a consequence of the cells' failure to synthesize oligo(ADP-Ribose).

Significance for Cancer Research: (NCP Objective #6, Approach #3). Understanding the mechanisms whereby cell progression through the cell cycle is controlled may suggest ways in which cancer cells can be reversed to a non transformed state, or killed. Studies of oligo(ADP-Ribose) synthesis may be a good model system for studying the transition from one growth stage to an-

other. Synthesis of the oligomer occurs at a restricted time in the cell cycle and blockage of synthesis also blocks cell division.

Proposed Course of Research: The major effort during the coming year will be toward further characterization of the acceptor protein. As an indication of its function, the purified protein will be stripped of its bound oligo(ADP-Ribose) and the protein with and without oligomer will be compared for its ability to bind to DNA-cellulose columns. These studies will be important in evaluating the possibility that chromatin structure is altered by the modification reaction. Preliminary evidence indicates that the same oligoADP-ribosylation of protein seen in vitro with human cells also occurs in vivo. Further effort will be directed toward isolating and quantitating the proteins which are oligoADP-ribosylated in intact human cells during various stages of the cell cycle.

Publications:

1. Smith, G. H., Kidwell, W. R., and Schneider, W. C.: Cytoplasmic DNA in rat corpoa lutein cells: Effect of prolactin on ^{3}H-thymidine incorporation. Exptl. Cell Res. 96: 321-334, 1975.

2. Kidwell, W. R. and Mage, M. G.: Changes in poly(adenosine diphosphate-ribose) and poly(adenosine diphosphate-ribose) polymerase in synchronous Hela cells. Biochem. (in the Press).

Patents:

1. Knazek, R. A., Gullino, P. M., Dedrick, R. L., and Kidwell, W. R.: Cell culture on semi-permeable tabular membranes. Patent No. NR325210 (Austria), 1975.

2. Knazek, R. A., Gullino, P. M., Dedrick, R. L. and Kidwell, W. R. Cell culture on semi-permeable tabular membranes. Patent No. 564086 (Switzerland), 1975.

3. Knazek, R. A., Gullino, P. M., Dedrick, R. L. and Kidwell, W. R. Cell culture on semi-permeable tabular membranes. Patent No. 987281 (Italy), 1975.

4. Knazek, R. A., Gullino, P. M., Dedrick, R. L. and Kidwell, W. R. Cell culture on semi-permeable tabular membranes. Patent No. 1395291 (Great Britain), 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05216-05 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Role of Cyclic Nucleotide on In Vivo Tumor Growth Inhibition.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yoon Sang Cho-Chung	Research Chemist	LPP, NCI
OTHERS:	Timothy Clair	Chemist	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 1/4	1	1 1/4

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: To understand the mechanism by which cyclic nucleotide produce tumor growth inhibition in vivo. One specific focus is to assess the qualitative analysis of cAMP-binding proteins in relation to cAMP-regulatory function (DBcAMP-"responsiveness" in vivo).

PHS-6040
(12-75)

Methods: i) Tumors: Two cell populations isolated from Walker 256 (hormone-independent mammary carcinoma), R3230 AC (hormone-responsive mammary adenocarcinoma) and hepatoma 5123 by selective transplantation; one regresses (DBcAMP-responsive) and the other continues to grow (DBcAMP-unresponsive) under DBcAMP treatment, were used. Also, primary, N-nitrosomethylurea-induced (NMU) and transplantable MTW9 mammary carcinomas were used. ii) DBcAMP(dibutyrylcAMP) was injected 10 mg/0.2 ml NaCl/day/200 g rat s.c. iii) cAMP assay: cAMP levels in tumors were measured by Gilman's competitive protein binding assay. iv) cAMP-binding assay: a) standard assay: cAMP binding activity was measured by the competition assay of Gilman at pH 4.5 or 6.5. b) total binding assay: the competition assay of Gilman was performed at 23° for 3 hr. (^{3}HcAMP binding to proteins includes not only free sites but also endogenously bound sites by the exchange). v) protein kinase assay: the activity was determined by measurement of the incorporation of ^{33}P from α-labeled ATP into histone. vi) cell fractionation and sucrose density gradient centrifugation followed standard techniques.

Major Findings:
1) Characterization of cAMP-binding proteins and protein kinase from DBcAMP-responsive and -unresponsive Walker 256: An extensive Scatchard analysis of cAMP binding by responsive and unresponsive cytosol showed that there is an apparent difference in the shape of binding curve and maximum binding sites between responsive and unresponsive tumors and that the difference seems to be due to pH stability difference of the binding proteins. The qualitative difference of binding proteins from responsive and unresponsive tumors also indicated in different heat stability of the binding proteins. Preincubation at 50° for 15 min., decreased the specific activity of higher affinity binding to proteins from unresponsive tumor by about 50%, but there was little or no effect on the binding proteins from responsive tumor. Unresponsive tumor possessed 2 fold lower cAMP-dependent protein kinase activity than responsive tumor. On the other hand, the catalytic properties of the enzyme in the absence of cAMP was similar between responsive and unresponsive tumors. The modification of cAMP binding proteins is, therefore, probably associated with the decrease in cAMP-dependent protein kinase activity of unresponsive tumor.
2) cAMP binding and protein kinase activities of DBcAMP-responsive and unresponsive Walker 256 in response to DBcAMP treatment in vivo: DBcAMP injections for 6 days resulted in a 50% decrease in total cAMP-binding and protein kinase activities of responsive cytosol. This depletion of cytoplasmic binding and kinase activities accompanied by a 3 fold increase of nuclei-associated binding and kinase activities. On the contrary, the binding and kinase activities of unresponsive cytosol and nucleus did not change and remained low during DBcAMP treatment. Cytosol from unresponsive control tumor showed an altered sedimentation pattern of cAMP-binding and protein kinase activities from that of responsive cytosol. The sedimentation profiles of cAMP-binding and protein kinase of control nuclei from both responsive and unresponsive tumors showed small broad peaks. In contrast, the nuclear extract from DBcAMP treated responsive tumor gave a sharp sedimentation profile composed mainly of a single species exhibiting both cAMP-binding and cAMP-independent protein kinase activities. This increase in nuclear cAMP-binding proteins and protein

kinase species was accompanied by a significant diminution of the macromolecular species in the cytoplasm. This finding suggests that the nuclei-associated peak of cAMP-binding and the kinase activity found in the DBcAMP-treated responsive tumor may have originated from the cytosol. The nuclear accumulation of cAMP-binding and protein kinase species, however, was not found in the DBcAMP-treated unresponsive tumor. Thus a correlation was found between nuclear accumulation of both cAMP-binding protein and protein kinase, and tumor regression due to exogenous DBcAMP.

3) A parallel between heat stable cytoplasmic cAMP-binding proteins, nuclear accumulation of both the binding proteins and protein kinase, and arrest of growth due to exogenous DBcAMP: A relationship between heat stability of cAMP-binding proteins in tumor cytosol and DBcAMP-"responsiveness" in vivo was shown with 4 types of rat tumors (see tumor models). The binding proteins from tumors growing under DBcAMP treatment (DBcAMP-unresponsive) were more sensitive to temperature than those from tumors regressing after DBcAMP treatment (DBcAMP-responsive), thus confirming the findings with W256 lines. The high affinity binding capacity of the unresponsive tumors was reduced to 50% or less when the cytosol was pre-incubated at 50°C for 15 min.; under the same treatment, more than 75% of the binding capacity of the responsive tumors was retained. This a striking parallel was shown between heat stable cytoplasmic binding proteins and DBcAMP-induced tumor regression in vivo with the exception of 8 tumors out of 52 tested. The finding with Walker 256 that a close parallel between the heat stable cytoplasmic cAMP-binding proteins and their intracellular redistribution during DBcAMP treatment was reproduced with DBcAMP-responsive and -unresponsive R3230AC and hepatoma 5123. Only the binding protein-cAMP complex in the DBcAMP-responsive tumor cytosol showed the capability of nuclear translocation during DBcAMP treatment; this event correlated closely with a simultaneous increase of nuclear protein kinase activity.

Significance to Cancer Research: (NCP Objective #6, Approach #3). These studies contribute to the understanding of the mechanism of cyclic nucleotide-induced tumor regression. A striking parallel between heat stable cytoplasmic cAMP binding proteins, nuclear accumulation of both the binding proteins and protein kinase, and arrest of growth due to exogenous DBcAMP implicate the important role of cAMP-binding proteins in growth control. The heat stability test of cytosol cAMP-binding proteins, thus, appears to be a promising diagnostic probe to predict DBcAMP responsiveness in vivo.

Proposed Course of Research: To extend the investigation on the mechanism of cAMP action on growth control, the following proposal is made:

i) Extend the heat stability test of cytosol cAMP-binding proteins in human tumors;

ii) Assess whether failure of nuclear accumulation of cAMP-binding in DBcAMP-unresponsive tumor protein is due to defective cytoplasmic binding protein or due to defective nuclear acceptor sites;

iii) Assess whether nuclear acceptor sites are located in the acidic protein or histone of chromatin;

iv) Assess the interrelationship between cAMP-binding protein and protein

kinase in nuclei;
v) Assess whether phosphorylation is intimately related with tumor regression.

Publications:

1. Cho-Chung, Y.S., and Clair, T.: The role of cAMP in neoplastic cell growth and regression. III altered cAMP-binding in DBcAMP-unresponsive Walker 256 mammary carcinoma. Biochem. Biophy. Res. Commun. 64: 768-772, 1975.

2. Cho-Chung, Y.S.: Role of Cyclic Nucleotides on *in vivo* Tumor Growth Inhibition. In: Second International Congress on Pathological Physiology, Praha, Czechoslovakia, July 8-11, 1975 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CBD 05219-05 LPP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vitro Simulation of Hormone-dependent Mammary Tumor Regression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Knazek	Senior Investigator	LPP	NCI
Other:	P. M. Gullino	Chief, Lab. of Pathophysiology	LPP	NCI
	S. Chen Liu	Chemist	LPP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1/2	1/2

SUMMARY OF WORK (200 words or less - underline keywords)

Summary: A bundle of tube shaped semi-permeable membranes, when perfused with tissue culture medium simulates the in vivo capillary bed. Isolated cells injected into the extracapillary space receive nutrients and have cell products removed by diffusion through the capillary walls. In this nearly physiologic environment the cells form solid masses of tumor. The effect of various hormones on these in vitro solid tumors is being studied to determine the mechanisms of hormone-dependent tumor growth and regression. Specific hormone receptors are being studied and isolated to complement these studies.

Objectives: To reproduce and study in vitro, the hormone-dependent regression process of mammary tumors observed in vivo.

Methods Employed: Established cell lines placed within artificial capillary beds from solid tumor masses in vitro. The capillary network, perfused by tissue culture medium, maintains the stability of the pericellular microenvironment while providing nutrient support and waste product removal for the cells. Our previous studies have shown that when capillary membranes permeable to 100,000 MW substances are mixed with silicone polycarbonate capillaries, the required macromolecular transport and oxygenation are provided to the cells. This technique provides the most physiologic conditions yet obtainable for cell growth in vitro.

MCF-7 is an established, cloned cell line of mammary carcinoma that responds to hormonal manipulations of monolayer cultures in vitro.

An assay for prolactin and growth hormone receptors has been developed using a modification of the Shiu-Friesen method to determine the ability of cell membranes to bind polypeptide hormones. In addition, affinity chromatographic techniques have been adapted to purify these receptors from the other membrane components by dissolving membranes in detergent, then adsorbing and subsequently eluting them from a human growth hormone (hGH) - affinity chromatography column.

Snell dwarf mice are autosomal recessive mutants whose circulating levels of growth hormone and prolactin are lower than their phenotypically normal littermates, and who, consequently, attain only 1/4 of the normals' weight.

Major Findings: MCF-7 cells formed large solid masses, filling the extracapillary space of the culture units within four weeks. Histologic studies of these masses showed that the cells had organized into acinar and ductular structures when medium with charcoal adsorbed serum was supplemented with insulin, human placental lactogen, and estrogen. Electron microscopy revealed the presence of desmosomes between adjacent cells. If estrogen, insulin, and human placental lactogen were not added to the perfusion medium, a slight disordering of this cellular organization occurred within 3 days and a three-fold decrease in the amount of ^{3}H-TdR uptake was observed.

Liver membranes of the phenotypically normal littermate of the Snell-dwarf mouse bound hGH with an affinity constant 11×10^{9} M^{-1} and having 38 fmole binding sites/mg protein while the liver of a pregnant female had approximately twice the number of binding sites/mg protein. The liver of the homozygous dwarf, however, did not bind hGH. When the dwarf animal was treated with daily intraperitoneal injections of 100μg hPL, binding of hGH was induced, being noted first at 2 weeks after the initial injection and reaching 2/3 of the normal levels at 3 weeks. Cessation of hPL injections permitted the levels of binding to decay to 20% of peak levels after 9 days.

Livers excised from female rats were pulverized in liquid nitrogen, homogenized, and dissolved in Triton-X. The 100,000xg supernatant of this suspension contained the solubilized hGH-receptor as demonstrated by specific binding to ^{125}I-labeled hGH. After this receptor solution was adsorbed to an hGH affinity column, the purified liver hGH-receptor was eluted by highly concentrated $MgCl_2$ solutions and subsequently dialyzed to remove the $MgCl_2$. The fractions containing the highest binding activity have been injected into rabbits in an attempt to develop an antibody to the hGH receptor.

The same procedure has been applied to purify the hGH receptor from lactating rabbit mammary glands. The active, purified fractions were injected intradermally with complete Freunds adjuvant into 25 locations on the backs of female rats in an attempt to induce antibodies to the lactogenic receptor. Booster injections were administered 3 weeks later when the animals were inoculated with the prolactin/growth hormone producing MtTW10 tumor and the hormone dependent MTW9 mammary carcinoma. Both tumors grew at the same rates and the circulating levels of prolactin were indistinguishable from the control animal levels. However, the livers of four of five animals treated with the receptor injections had hGH receptors exhibiting $K_a = 1.1 \times 10^9 M^{-1}$ and 770-910 femtomoles binding per mg protein. The livers of control animals bound hGH with $K_a = 0.4$, 0.3, and $1.5 \times 10^9 M^{-1}$ in the amounts of 2200, 2100, and 770 femtomoles per mg protein. These initial experiments indicate that formation of antibodies toward hGH receptors may be induced in rats and thereby alter the ability of the liver to bind this lactogenic/somatotrophic hormone.

Significance for Cancer Research: (NCP Objective #4, Approach #1). The environment which artificial capillaries provide is more physiologic than that of any other type of cell culture. This allows isolated cells to form solid tumor masses in vitro and to exhibit behavior that more closely resembles that of the in vivo state. Thus, solid mammary tumors may be formed in vitro and the hormone-dependent processes can be studied without uncontrollable in vivo factors.

Production of anti-hGH receptor antibodies within an experimental animal bearing a prolactin/growth hormone dependent tumor might induce an immune-based regression by blocking the effect of the circulating hormones on the tumor. This would then provide an alternative to the surgical procedures for endocrine ablation and serve as an animal model for the study of such clinical manipulations in patients afflicted with hormone dependent neoplasms.

A heterologously derived antibody directed toward the hGH receptor of experimental animals would provide the means for detecting inactive receptors of various tumors and tissues by radioreceptor or fluorescent techniques. The formation or activation of these receptors and the factors that influence the same can then be studied both in vivo and in vitro.

The induction of hGH-receptors in the livers of Snell dwarf mice may provide a model by which prolactin receptors develop within mammary tumors.

Proposed Course of Research: Various combinations of steroid and polypeptide hormones will be used to study the formation of organized structures within the solid MCF-7 mammary carcinomata grown within the artificial capillary system. Attempts will continue to induce homologous antibodies in rats against lactogenic or somatotrophic receptor sites to alter their character and perhaps induce regression of the hormone-dependent tumors. Sera of rabbits previously injected with purified, solubilized rat liver hGH-receptors will be assayed to determine if anti-hGH receptors have been formed. This will be done by 1) iodination of the igG formed and subsequent radioimmunoassay of tissues known to contain the receptor, 2) inhibition of hormone binding by pre-incubation of receptor tissues with sera, 3) fluorescent antibody techniques.

Publications:

1. Knazek, R. A. and Skyler, J. S.: Production of Polypeptide Hormones by Cells Cultured on Artificial Capillaries. Proceedings of the International Symposium on Growth Hormone, Milan, Italy, 1975. Excerpta Medica, Amsterdam, The Netherlands (In Press).

2. Knazek, R. A. and Skyler, J. S.: Secretion of Human Prolactin in Vitro. Proceedings of the Society for Exptl. Biol. and Medicine (In Press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 05221-03 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Comparison of nucleoproteins of normal animal tissues with neoplastic tissues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Rotherham Research Chemist LPP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Nucleic Acids Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

To discover if there are changes in nucleoprotein structure that are basic to neoplastic change and to characterize them.

PHS-6040
(12-75)

Project Description:

Objectives: To discover if there are differences in the structures, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with respect to comparable tissues that are becoming neoplastic.

Methods Employed: Suitable chemical agents will be used which enhance the occurrence of neoplastic changes in mouse and/or rat tissues. Nuclear fractions will be isolated and examined for possible differences in metabolic modifications of the nucleoproteins of tissues of treated and control animals.

Major Findings: Beyond that given in the report on this project for July 1, 1974-June 30, 1975, no new data have been obtained.

Significance for Cancer Research: (See objectives). National Cancer Program Plan Objective and Approach: Cause and prevention; nature and rate of oncogenic cell transformations in carcinogenesis (3, 2).

Proposed Course of Research: A critical and operationally difficult aspect of this investigation is the isolation from epidermis of pure and unaltered component nucleoprotein or protein fractions that are known to have been located in the nucleus. Satisfactory methods for accomplishing this within the limitation of resources available to the laboratory have not yet been developed. The project itself is acknowledged to be clearly relevant to investigation of biochemical modifications which may be characteristic of neoplastic changes. It is sound in design. Also it is within an area of biochemical research from which it is very well possible that data significant for the overall objective may be obtained. It would be helpful to the progress of the study if the investigator's requests for equipment and supporting assistance were respected, dealt with fairly and summarily and if the working atmosphere were reasonably conducive to creative effort. Work continues in the direction indicated in the previous report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08205-05 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Application of the Artificial Capillary Culture Technique to Polypeptide Hormone Production

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Knazek	Senior Investigator	LPP, NCI
OTHERS:	P. M. Gullino	Chief, Laboratory of Pathophysiology	LPP, NCI
	Jay S. Skyler	Research Assoc., Hypertension-Endocrine Branch	NHLI

COOPERATING UNITS (if any)
Experimental Therapeutics Branch, NHLI

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION
Cell Cycle Regulation

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1/2	1/2

SUMMARY OF WORK (200 words or less - underline keywords)

The artificial capillary cell culture technique provides a pericellular microenvironment that closely resembles the in vivo state. This may allow normal cells to maintain their differentiated functions for prolonged periods of time in vitro. Cells are maintained in a physiologic state without many of the artifactual conditions imposed by standard methods of tissue culture. The technique is, therefore, especially well suited for the culture of cells of endocrine origin. Response of the cells to various physiologic agents are being studied as well as the secretion of various hormones over long periods with the possible application to continuous production of hormones for laboratory and clinical uses.

Project Description:

Objectives: Establishment of a human pituitary cell line that produces prolactin or growth hormone. Development of a method that provides both large quantities of pituitary hormones and a means for studying their synthesis and secretion.

Methods Employed: Human pituitary tumors and human fetal pituitaries are mechanically and/or enzymatically dispersed into single cell suspensions and either placed in monolayer culture or in the extracapillary space of an artificial capillary culture unit. The media are replaced periodically and then characterized by radio-immuno dilution and radio-receptor dilution studies, and polyacrylamide gel electrophoreses (PAGE). If the dilution studies produce curves parallel to those of hormone extracted from glands and purified to homogeneity, then they are indistinguishable. PAGE studies provide retardation coefficients which when plotted against gel concentrations yield Ferguson plots which in turn provide information on the charge and size of the molecule. The statistical significance of these values are then expressed as K_r-Y_o ellipses; overlapping ellipses indicates that the two electrophoresed substances are indistinguishable.

Modification of the artificial capillary perfusion circuit into a single pass mode of operation enables pituitary cells inoculated into the extracapillary space to be pulsed with pharmacologic or physiologic agents. The influence of these substances on the kinetics of secretion may then be studied under conditions that approach those of the in vivo state.

Major Findings: A human pituitary tumor continued to secrete prolactin over a 6 month period while maintained within an artificial capillary culture unit. The hormone was compared to human prolactin (hPRL) standard obtained from the National Pituitary Agency (NPA) by extraction of normal autopsied pituitaries. The tissue culture-derived hPRL and standard hPRL were indistinguishable by gel filtration, radio-immuno dilution studies, and Kr-Yo ellipses of PAGE. Human growth hormone (hGH) obtained from monolayer cultures was likewise indistinguishable from standard NPA hGH by gel filtration, PAGE Kr-Yo ellipses, radioreceptor dilution, and in one of three radio immunodilution assays. These findings indicate that hPRL and hGH produced by tissue culture methods may serve as alternative sources for these hormones for future laboratory and clinical studies. Although this project has previously shown that more than 3 mg hPRL could be obtained from cells in a single capillary culture unit over a 4 month period, our recent studies indicate that only .4 mg of hGH was secreted over a 79 day period when cultured in the artificial capillary network. Unknown trophic factors are lacking in the perfusion medium, perhaps hypothalamic, to promote hGH secretion at high rates in vitro.

Rat pituitaries maintained in the artificial capillary culture units for 7-10 days continue to secrete prolactin (rPRL) and growth hormone (rGH). If perfused with fresh medium in the single pass mode of perfusion and the perfusate analyzed by radioimmunoassay, a basal level of rPRL secretion is reached. When various fractions of rat hypothalamic extract are dissolved in aliquots of medium and used to perfuse the capillary bed in 15 min bursts, significant

increases or decreases in rPRL secretion are observed depending on the fraction. The increases observed are undoubtedly due to thyrotropic releasing hormone whereas the decrease in rPRL secretion is thought to be a result of the prolactin inhibiting factor (PIF), as yet unidentified.

Significance for Cancer Research: (NCP Objective #3, Approach #3.) The hPRL and hGH obtained from in vitro tissue cultures have been shown to be indistinguishable from the standard hormones obtained by extraction of autopsy pituitaries. The decreasing frequency of autopsies and increasing demand for these hormones have limited their availability. Human pituitaries cultured within artificial capillary beds may therefore provide significant amounts of prolactin or other hormones for studies of hormone dependent breast cancer.

The characteristics of the capillary culture unit enable the study of rapid responses to both stimuli and inhibitors of cell function. Application of this technique may enable isolation of the prolactin inhibiting factor within the hypothalamus. Identification of PIF and its subsequent synthesis could provide an agent which when administered to patients might physiologically inhibit prolactin secretion in patients with breast cancer. In effect, this could be viewed as a trial hypophysectomy by medical means to determine the effect on the patient without subjecting them to hypothysectomy.

Proposed Course of Research: Fetal and neoplastic adult pituitaries will be placed in both monolayer culture and in artificial capillary culture in an attempt to establish a cell line that secretes hGH. Various methods of cell dispersion, types of media, hormonal supplements, and viral transformation will be used to this end.

The initial studies using fractionated rat hypothalamic extract to stimulate and inhibit rPRL secretion in the capillary culture units will be repeated. If the PIF component is isolated, its amino acid sequence will be determined.

Publications:

1. Knazek, R. A. and Skyler, J. S.: Secretion of Human Prolactin in vitro. P.S.E.B.M. (in press) March 1976.

2. Knazek, R. A. and Skyler, J. S.: Production of Polypeptide Hormones in Tissue Culture. Proceedings of the Third International Symposium on Growth Hormone and Related Peptides, 1975. Excerpta Medica, Amsterdam, The Netherlands (in press).

3. Skyer, J. S., Lovenberg, W., Rogol, A.D., and Knazek, R. A.: Characterization of Growth Hormone and Prolactin Produced by Human Tumors in Culture (in referee).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08209-02 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Synthesis and Processing of Heterogeneous Nuclear RNA in Non-dividing Human Lymphocytes and Modifications Related to Stimulation of Cell Growth

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shelby L. Berger	Research Chemist	LPP, NCI
OTHERS:	Herbert L. Cooper	Head, Cellular and Molecular Physiology Section	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	2/3	0

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to characterize the synthesis and processing of heterogeneous nuclear RNA, thought to be the precursor to messenger RNA, in physiologically non-dividing human cells. Points in the metabolic sequence which may be involved in control of normal processing will be sought. The relation between such biochemical controls and modifications in the use of messenger RNA related to growth stimulation will be sought. Comparisons will be made between the functioning of these regulatory steps in normal and neoplastic lymphoid tissues.

PHS-6040
(12-75)

Methods: Routine procedures used throughout.

Major Findings: The synthesis of large hnRNA molecules in the nucleus is believed to occur in three steps. First the 5' portion of the molecule is completed presumably by transcription from the DNA template by a DNA-dependent RNA polymerase, then a sequence of up to 200 adenylate residues is added by an adenylating enzyme and finally a large portion of the 5' end of the molecule is degraded before the finished poly(A)- bearing mRNA molecule is transported to the cytoplasm.

In resting lymphocytes, the largest hnRNA molecules are poorly represented in the pool of poly(A)-bearing hnRNAs. Molecules with poly(A) "tails" are found predominantly among the intermediate sized hnRNA molecules. Whereas hnRNA is turned over rapidly, the addition of polyadenylate to the 3' end confers a marked stability. In the present study such hnRNA(+)poly(A) molecules decayed with a half life of 7 hours. During the processing period the size of most of the surviving molecules remained unchanged. However after 7 hours a small pool of cytoplasmic sized hnRNA(+)poly(A) was detected in the nucleus by gel electrophoresis. No intermediate sized molecules were found. These data indicate that processing of hnRNA(+)poly(A) to poly(A)-bearing mRNA occurs rapidly; once a given poly(A)-bearing hnRNA is selected for maturation, the shortening reaction(s) proceeds quickly to completion and the product is immediately transported to the cytoplasm.

When the kinetics of processing of hnRNA(+)poly(A) were compared with those of mRNA (described in Project #08212) it became evident that approximately 75% of newly synthesized hnRNA(+)poly(A) could be found in the nucleus at a time when no further transport to the cytoplasm could be observed. These data strongly suggest the existence of two processing pathways for hnRNA(+)poly(A) drawn from a common pool or two pools of hnRNA(+)poly(A) which the cell handles differently. One class is destined for immediate maturation and rapid export to the cytoplasm while the second class probably decays slowly in the nucleus but may be exported very slowly to the cytoplasm.

Significance for Cancer Research: This project conforms to Objective #3, Approach #1 of the National Cancer Plan. It aims at an understanding of the biochemical control mechanisms by which normal cell growth and function are maintained. The causes of disordered cell growth and activity in neoplasia may then be better understood and rational attempts made to prevent or modify them.

Proposed Course of Research: Further characterization of the two classes of hnRNA(+)poly(A) is anticipated. The half life of bulk poly(A)-bearing hnRNA will be reexamined in the hopes of finding rapidly disappearing molecules. Inhibition of RNA synthesis will be employed in order to sharpen the kinetics of mRNA processing. Then examination of changes in mRNA processing related to cell growth stimulation will be studied. Following this, a comparison will be made between growing lymphocytes from normal human peripheral blood and from neoplastic lymphoid cells. The latter will include long term lymphoid

lines (Burkitt lymphoma, e.g.) of both B- and T-cell types; patient material from chronic lymphocytic leukemia, lymphomatous lymph node cells, and, if possible, acute lymphatic leukemia.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 08210-02 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Behavior of Ribosomes, Polysomes and Ribosomal Subunits in Non-dividing Human Cells and Changes Related to Onset of Normal Cell Growth.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert L. Cooper	Head, Cellular and Molecular Physiology	LPP, NCI
OTHER:	Shelby L. Berger	Research Chemist	LPP, NCI
	Richard Braverman	Chemist	LPP, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION
Cellular and Molecular Physiology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1/2	1/2

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: To characterize the behavior of ribosomal particles in a non-growing cell population in which the rate of protein synthesis is maintained at low levels; to correlate such behavior with the limitation in protein synthesis so as to understand the mode of translational control employed by the cell in adjusting protein synthesis to growth and metabolic activities; to determine the regulatory steps which are operative in the enhancement of protein synthesis associated with the onset of cell growth.

PHS-6040
(12-75)

Methods: In addition to routine methods, techniques were developed to permit examination of ultraviolet absorbancy profiles of ribosomes and polysomes from resting and growing human lymphocytes analyzed by ultracentrifugal sedimentation in sucrose gradients. Because of limited amounts of material, and the small size of lymphocytes, this methodology has previously never been successfully applied to human peripheral blood lymphocytes. We overcame these problems by a combination of optimizing lymphocyte yields from human peripheral blood, and a detergent cell lysis method which gave maximum yields of cytoplasm while minimizing degradation of ribosomes.

Major Findings: Unlike growing cell systems studied by most investigators, physiologically resting lymphocytes were found to maintain the great majority (about 70%) of their cytoplasmic ribosomes as single 80s ribosomes, (monomers). In addition, significant numbers of unassociated (native) 60s and 40s, subunits were found. The monomers were found to consist of a major fraction (80%) of inactive particles (easily dissociable in high salt, "free ribosomes") and a minor fraction of active particles (monosomes). A major portion of the protein synthesis of the resting lymphocyte was found to occur on such monosomes (i.e. - a single ribosome traversing a mRNA), while free ribosomes did not engage in protein synthesis. Progressively decreasing numbers of larger polysomes were found. The number of native subunits was large relative to the numbers of polysomes. The large accumulation of free ribosomes (i.e. - associated subunits inactive in protein synthesis) was shown to be a real feature of the intact lymphocyte and not an artifact due to association of large pools of free subunits during technical procedures. Thus, the low rate of protein synthesis in resting lymphocytes is correlated with:

1). Maintenance of most of the cell's available ribosomes in an inactive state (free ribosomes).

2). Performance of protein synthesis largely on monosomes and smaller polysomes, with inefficient use (i.e. - unsaturation) of available mRNA.

Labeling studies with [^{3}H] uridine revealed that new rRNA first entered native 40s and 60s subunits and polysomes (including monosomes). After a delay of 15-20 minutes particles began to enter the free ribosomes. A number of pulse-chase experiments established that free ribosomes did not arise directly from new synthesis, but were converted from native subunits and particles in monosomes and polysomes as those components aged. Particles in free ribosomes seldom returned to protein synthesis. It was estimated that a new ribosome was used 10-15 times in protein synthesis before being inactivated as a free ribosome.

Physical and functional differences between free ribosomes and native subunits were observed. Native subunits remain dissociated (60s and 40s subunits) at both high and low ionic strength, while subunits of free ribosomes (derived subunits) associate at low ionic strength but dissociate at high ionic strength. Native 40s subunits sediment slightly more slowly than derived 40s subunits. This may be due to absence of one or more proteins

from the derived subunit. The change in association behavior and sedimentation characteristics may thus be explained by loss of ribosomal proteins, which normally maintain dissociation, when native subunits are converted to free ribosomes. This modification effectively removes the particle from participation in protein synthesis, since native subunits remain associated as 80s ribosomes in the cell. Dissociation into subunits is required for re-entry into protein synthesis, and this is apparently restricted in resting lymphocytes.

Analysis of distributions of particles in various classes has indicated that, while the resting lymphocyte tends to sequester its ribosomes in a large pool of inactive particles, this feature is not rate-limiting for protein synthesis. It now appears more likely that an early step in initiation of native subunits, due either to an inhibitor of initiation or to a deficiency of an initiation factor, is rate limiting on protein synthesis in the resting cell. Abundant mRNA appears to be present, which is underutilized.

Studies of the early stages of growth activation in lymphocytes have revealed that the undissociated, inactivated free ribosome pool was dissociated and activated to enter protein synthesis as an early step in growth stimulation. It was established that virtually all of the increase in protein synthesis characteristic of the initial 20 hours of growth stimulation can be carried out by activating pre-existing free ribosomes. Synthesis of new ribosomes was not essential, although such synthesis is normally highly stimulated. Thus, the utility of the large pool of inactive ribosomes maintained by the resting cell may be to provide the particles necessary to mount an effective response to a physiological stimulus under pathological conditions which might not provide the necessary nutrients needed for synthesis of new ribosomes; i.e. - chronic inflammation, anoxemia, reduced circulation, etc.

Surprisingly, it was found that the initial steps in the activation of free ribosomes did not require synthesis of new proteins, despite the evidence of a difference in protein content between native and derived subunits. This implies that the necessary proteins for binding to free ribosomes in order to enhance their dissociation are already present in the cell but not utilized. Apparently an early step in growth stimulation is the activation of these proteins, by a process which is now being studied.

Of further interest was the finding that the activation of free ribosomes was prevented by conditions which inhibited production of new mRNA. This is in contrast to the evidence that there is no shortage of mRNA in the resting cell. Since new protein synthesis is not required to activate free ribosomes, it is not clear what role the new mRNA might play. We are currently exploring the possibility that not mRNA, but another species of RNA inhibited by the same conditions - possibly tRNA - is the essential component.

Significance to Cancer Research: By the elucidation of the mechanisms which regulate protein synthesis in resting cells and during a physiological growth

sequence, we hope to provide the basis for recognition and understanding of derangements in these controls which may characterize malignant transformation. This objective supports Objective 3-1 of the NCP.

Proposed Course of Research:

1). Further characterization of the basis for inactivation and re-activation of ribosomes in resting lymphocytes.

2). Examination of sequential events in this system during the return to resting state which occurs in vitro following stimulation by PHA.

3). Investigation of the possibilities for influencing control steps which have been elucidated by the use of drugs or other approaches. In vitro studies only at this stage.

4). Comparison of normal and neoplastic lymphoid cells with respect to these control mechanisms.

Publications: Free Ribosomes in Physiologically Nondividing Cells: Human Peripheral Lymphocytes: H. Cooper, S. Berger, R. Braverman. Submitted to J. Biol. Chem. (Will be in press at end of this review period).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08211-02 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Detection of Specific Messenger RNAs in Resting and Growing Human Lymphocytes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert L. Cooper	Head, Cellular and Molecular Physiology	LPP, NCI
OTHERS:	Shelby L. Berger	Senior Staff Fellow	LPP, NCI
	Richard Braverman	Chemist	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cellular and Molecular Physiology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/4	1/2	1/4

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: To determine whether mRNAs for specific products synthesized only by growing lymphocytes are present but untranslated in resting lymphocytes; to determine whether such sequences are present in the nucleus, cytoplasm, or both.

It is hoped, by this approach, to clarify the level of control of the source of mRNA for specific proteins, and the way in which growth stimulation affects these controls.

PHS-6040
(12-75)

Methods: RNA will be extracted from resting and growing lymphocytes (whole cells, cytoplasm, and nuclei). Messenger RNA will be separated by affinity chromatography on oligo deoxythymidylate cellulose; mRNA will then be injected into amphibian oocytes. Proteins synthesized by injected and control oocytes will be examined for activity of specific proteins. Initially, assays will be performed for human interferon. This protein is produced only by growing lymphocytes, and can be assayed in minute amounts. Since it is species specific, no interference from amphibian proteins is anticipated.

Major Findings: Because of activity in other areas, only a small amount of effort was devoted to this project. A cell-free protein synthesizing system from wheat germ was prepared and shown to translate globin mRNA in vitro. This system will be used to establish the overall mRNA activity of various RNA preparations from lymphocytes. Initial experiments were performed to develop the technique of injection of mRNA into frog oocytes in order to translate specific mRNAs into active products. Contact has been made with NIAMD (Dr. Robert Friedman) in whose laboratory assays for interferon activity will be performed. Since lymphocytes, when stimulated to grow, synthesize large amounts of interferon, it is hoped to establish the means of control of availability of mRNA for this product: is control at the level of transcription, mRNA processing and transport, or translation?

To date, RNA preparations from lymphocytes have yielded little mRNA activity. This is undoubtedly related to the levels of RN'ase in lymphocytes, which damages the mRNA as it is geing extracted. Efforts are now being directed toward improving RNA preparation methods to overcome this problem.

Significance to Cancer Research: Supports Objective 3-1, 3-2, and 3-3 of NCP.

Proposed Course of Research: As indicated above.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08212-02 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Control of Messenger RNA Metabolism in Human Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Shelby L. Berger Research Chemist LPP, NCI

OTHERS: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cellular and Molecular Physiology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/2	1/2	0

SUMMARY OF WORK (200 words or less - underline keywords)

In this project the steps involved in the synthesis and degradation of messenger RNA and its precursors will be elucidated. The mechanisms employed by the cell in exercising control at key steps in mRNA metabolism will be studied. Experiments to devise methods for inhibiting or activating these processes in order to manipulate cell growth are anticipated.

PHS-6040
(12-75)

Objectives: To elucidate the steps involved in the synthesis and degradation of messenger RNA and its precursors; to determine the mechanisms employed by the cell in exercising control at key steps in mRNA metabolism and to devise methods of inhibiting or activating these processes in order to manipulate cell growth.

Methods: Routine procedures have been used throughout.

Major Findings: The lymphocyte is ideally suited for studies of the complex mechanisms by which cellular growth is regulated. When isolated from human peripheral blood, the lymphocyte is a non-dividing cell. It can be maintained in culture for short periods during which the control processes characteristic of the physiologically resting state can be examined. Such resting cells can subsequently be stimulated to enter a state of active proliferation by the addition of one of a number of mitogens to the culture medium. Therefore the regulatory mechanisms employed upon entering a state of rapid growth and returning to one of restricted cellular activity can also be studied in human lymphocytes.

The life times of messenger RNA, defined as cytoplasmic poly(A)-bearing RNA, were studied by incubating cells for short periods of time with radioactive RNA precursors, washing them and resuspending the cells in unlabeled medium. After a given period the amount of mRNA in the cytoplasm can be determined. The results of such pulse-chase experiments indicated that two classes of mRNA exist in resting lymphocytes, a labile class with a half life of 17 minutes and stable component which did not appear to be degraded during 24 hours of observation. Quantitation of the amount of each class among newly synthesized molecules revealed that 80% of the mRNA in the cytoplasm was rapidly degraded while the remainder entered the stable pool.

The size distribution of newly synthesized and old mRNAs in the cytoplasm were compared. On the average the former (which includes both labile and stable messages) had a slightly broader size distribution and a higher molecular weight than the stable variety. Since it is unlikely that shorter messages are per se more worthy of being conserved than the larger labile class, these findings can be interpreted as evidence for cytoplasmic processing of all mRNAs.

Messenger RNA lifetimes were also examined in lymphocytes during the transition from the nondividing state to one of rapid growth and also during the period of most active proliferation. In both cases, radioactive precursors continued to be incorporated into RNA for 6-8 hours after redispensing cells in unlabeled medium. Under such conditions the presence of labile mRNA is easily obscured by the relentless accumulation of more stable species. In order to circumvent this problem, pulse times were shortened, inhibitors of RNA synthesis and of polyadenylation were employed, culture conditions were manipulated and a variety of RNA precursor was examined. In all cases no labile messenger RNA molecules were observed; only the stable species with a minimum half life of 5-6 hours could be detected.

Our findings indicated that the control mechanisms operative in growing cells may differ from those in physiologically nondividing populations.

Significance for Cancer Research: Objective 2, #3; Objective 3, #1,3 and 4.

Proposed Course of Research: The basis for the classification of messenger RNAs into stable and labile categories shall be pursued in the near future. Stabilization of certain messages may be accomplished by several mechanisms among which are: (1) binding of the mRNA to a protective protein; (2) specific chemical modification of the RNA molecule itself; (3) combination with the protein synthesizing apparatus of the cell; (4) production of specific ribonuclease inhibitor(s). Each of these possibilities shall be investigated. If indeed there are mRNA protecting proteins, we shall characterize those associated with both stable and labile messages. This project will require the development of adequate methods to isolate RNA-protein complexes from lymphocytes without massive degradation of RNA. The sequences in both classes will be compared by hybridization studies and the encoded protein products studies. (See Project #08211.)

The role of cytoplasmic processing of messenger RNAs will also be examined. We hope to elucidate the nature of the shortening reaction, and perhaps identify the enzyme(s) involved. Since loss of adenylate residues from the 3' end of the mRNAs is insufficient to account for the observed change in molecular weight, the 5' end including the synthesis and removal of the putative GTP "cap" will be studied.

The study of labile and stable messenger RNAs in malignant lymphoid cells is anticipated.

Publications: Berger, S. L. and Cooper, H. L.: Very short-lived and stable mRNAs from resting human lymphocytes. Proc. Nat. Acad. Sci, U.S.A. 72: 3873-3877 (1975).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08213-02 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Glucose-6-Phosphate Dehydrogenase Isozymes in Growing and Regressing Rat Mammary Carcinomas.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jeffrey S. Bodwin	Surgeon	LPP, NCI
OTHERS:	Yoon S. Cho-Chung	Research Chemist	LPP, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

To study biochemical units related to growth and regression of experimental hormonally dependent mammary carcinomas which mimic human cancers. To investigate the mechanism of microsomal G6PD activity disappearance in DBcAMP induced regressing tumors which will elucidate the role of the cyclic AMP system in tumor regression. To study the correlations between tumor regressions induced by hormonal deprivation with that induced by DBcAMP treatment. Also to study the changes in G6PD activity and enzyme pattern during tumor regression and regrowth with estrogen injection and to correlate these findings with binding activity of estrogen and cAMP in the tumor cytosol and nucleus.

PHS-6040
(12-75)

Objectives: Previous work from this laboratory has shown that G6PD enzyme activity of rat mammary carcinomas exists in 4 enzyme forms located in mitochondrial, microsomal and cytosol cell functions. Characterization of these enzymes during growth, regression and DBcAMP treatment as well as correlations of this data with estrogen and cyclic AMP binding is underway to elucidate the role of these enzymes as biological markers in the early events of tumor regression.

Methods:
1) Primary 7, 12 DMBA induced and MTW9 transplantable rat mammary carcinomas both hormone dependent were used in studies.
2) G6PD activity of tumor cytosol was assayed directly with a spectrophotometer and histochemically by staining acrylamide gels after electrophoresis.
3) Partial purification of enzymes was performed with preparative disc electrophoresis.

Major Findings:
1) MTW9 carcinomas regressing after hormonal deprivation of the host show a decrease in total G6PD activity due to reduction in microsomal and soluble isozymes.
2) Treatment of MTW9 tumor bearing animals for 3 days with DBcAMP resulted in tumor growth arrest and microsomal G6PD activity disappearance. Cessation of treatment restores growth of tumor and microsomal activity.
3) Castration of 7, 12 DMBA tumor bearing rats results in progressive loss of soluble and particulate bound G6PD activity while reinjection with estrogen results in tumor regrowth and progressive reappearance of soluble and particulate enzymes.
4) Glucose-6-phosphate and galactose-6-phosphate serve as substrates for G6PD isozymes, but soluble and particulate isozymes demonstrate different K_m values for these substrates.

Significance to Cancer Research: (NCP Objective #6, Approach #3)
These studies provide insight into the biochemical events related to growth of experimental hormonally dependent mammary carcinomas which mimic human cancers. Understanding of the mechanism of microsomal G6PD activity disappearance in DBcAMP treated regressing tumor will elucidate the role of the cyclic AMP system in the control of abnormal growth.

Proposed Course of Research: To further investigate the molecular events of G6PD isozyme changes in relation to growth arrest and regression. Future objectives include:
1) determination whether DBcAMP active on G6PD activity is at transcriptional or translational level.
2) correlation of G6PD activity in growing and regressing tumors with estrogen and cyclic AMP binding activity.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08215-02 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Angiogenesis in Breast Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven Brem	Clinical Associate	LPP, NCI
OTHER:	Pietro M. Gullino	Chief, Laboratory of Pathophysiology	LPP, NCI

COOPERATING UNITS (if any)

1) Bethesda Naval Hospital

2) Surburban Hospital

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	1 1/4	2 3/4

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: To ascertain whether the ability of human mammary tissue heterotransplants to induce new formation of vessels can be utilized as a marker of neoplastic transformation.

PHS-6040
(12-75)

Methods: Biopsy specimens of human breast tissue are transplanted into the rabbit iris or cornea. Neovascularization is monitored with the slit-lamp stereomicroscope. The histology of the transplant is correlated to the angiogenetic capacity.

Major Findings: (1) The majority of human mammary carcinomas induce new formation of vessels in the rabbit iris. (2) Adipose tissue and stroma of the mammary gland bearing the tumor does not induce angiogenesis. (3) Fibroadenomas, cystic fibrosis and sclerosing adenosis have no angiogenic capacity. (4) Ductal papillomatosis has shown angiogenetic capacity.

As a consequence of these observations the work proceeds now in two directions: (a) collection of "atypical lobules" and introductal proliferative epithelium of human mammary glands and transplantation in the rabbit iris, and (b) systematic analysis of papilloma lines of mouse mammary gland with different potential for neoplastic transformation in the attempt to correlate angiogenesis and neoplastic transformation.

Significance to Cancer Research: The previous work with Dr. M. Gimbrone (see Report # NCI-8201) found a correlation for mouse mammary gland between the ability of hyperplastic tissue to induce angiogenesis and the onset of carcinoma. This work with Dr. Brem establishes that the same event probably occurs in humans, and suggests that the iris heterotransplant may offer a test for establishing whether mammary epithelium which appears hyperplastic under a standard histologic analysis has acquired the ability to induce angiogenesis. There is much evidence showing that a few neoplastic cells may grow to constitute a solid tumor only if they are able to elicit new formation of vessels from the host tissues. Demonstration of angiogenetic capacity may be a test for dilution of mammary carcinoma before histologic indications of infiltrative growth occurs.

Summary: Heterotransplants of human mammary tissue in the rabbit iris or cornea are used to study their ability of inducing new formation of vessels. Angiogenetic capacity of hyperplastic epithelium could be utilized as a test of early neoplastic transformation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08217-02 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Synthesis of Mammary Specific Proteins - Casein and α-Lactalbumin - in Mammary Carcinoma Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Pradman K. Qasba, Visiting Scientist — LPP, NCI

OTHERS: Pietro M. Gullino, Chief, Laboratory of Pathophysiology — LPP, NCI

Pran Chakrabartty, Visiting Fellow — LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	2/3	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To study the influence of hormone and growth conditions on the expression of mammary specific proteins - casein and α-Lactalbumin - in mammary carcinoma cells.

PHS-6040 (12-75)

Methods:
(1) Enzymatic method of detecting α-Lactalbumin activity.
(2) (a) Radioimmuno assay has been developed for detecting rat α-Lactalbumin and rat casein.
(b) Radioimmuno assay for human α-Lactalbumin, detecting 0.1 ng of human αLA, is used to determine amount of this protein in various human mammary tumor cell lines.

Major Findings:
(1) Levels of α-Lactalbumin in human tumor cell lines MDA-MB 231, MDA-MB 157, MDA-MB 134 and HBL-100 has been investigated. In the extracts made from MDA-MB cell lines, levels of αLA have varied from one batch of cells to another. Several times in some batches of cells we have not been able to detect any of it. In the extracts of HBL-100 cells we have observed more consistently an amount of αLA which ranged between 10-40 ng/mg of protein. This variability and unreproducibility in the levels of αLA in these cells may be the reflection of the physiological state of the cell.
(2) Rat mammary ascites tumors 13762 a and b cell lines and R3230AC showed 10 fold higher levels of αLA than virgin mammary glands by enzymatic method.

Proposed Course of Research: To determine the amount of rat α-Lactalbumin and casein in ascites mammary tumor cells 13762 and solid tumor R3230AC. Comparisons will be made between the cells grown in vivo and in vitro in the amounts of these proteins synthesized. Influence of the hormonal manipulations on the synthesis of these proteins in the cells grown in vivo and in vitro will be studied.

Significance to Cancer Research: By developing a very sensitive method for detecting α-Lactalbumin and casein one could characterize the functional differentiation of neoplastic mammary cells and correlate it with hormone dependence.

Publications: Abst. 1976 American Cancer Society meeting in Toronto.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08218-01 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Rat Lactose Synthetase: Purification and Properties of Rat-α-Lactalbumins.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pradman K. Qasba	Visiting Scientist	LPP, NCI
OTHERS:	Pran Chakrabartty	Visiting Fellow	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	2/3	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To purify and characterize rat milk whey proteins and rat casein for the studies on the regulation of the synthesis of these mammary specific proteins in normal and malignant rat mammary tissue.

PHS-6040
(12-75)

Methods: General methods for the purification of proteins.

Major Findings:
1) In rat milk 45-55% of the total proteins is casein. Individual caseins were purified to homogeneity on DEAE-cellulose column.
2) Two species of α-Lactalbumin were separated from rat milk. They were purified to homogeneity by gel filtration with Sephadex G-150 followed by DEAE-cellulose chromatography. The two exist in nearly equal amounts in rat milk. They differ, (a) in their net negative charge, (b) in their molecular weights (22,500 and 21,800 daltons), (c) in their interaction with rat galactosyl transferase. The low molecular weight α-Lactalbumin gave lower k_m for glucose than high molecular weight α-Lactalbumin with rat galactosyl transferase in lactose synthetase assay. Rat galactosyl transferase shows different apparent k_m for the two α-Lactalbumins. We at present do not know if the difference between the two α-Lactalbumins is in amino acid composition or in any modifications.

Proposed Course of Research:
1) Amino acid analysis of two α-Lactalbumins.
2) To test antibodies against two α-Lactalbumins for their cross reactivity.
3) Develop radio immuno assay for the two α-Lactalbumins (Work under progress).
4) Investigate if there are also two α-Lactalbumins in other species, e.g. in human or mouse milk.

Significance to Cancer: Characterization of milk proteins will help us to develop sensitive methods for detecting these proteins in neoplastic tissue.

Publications: The results of this study have been submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08219-01 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Control of Synthesis of Mammary Specific Proteins - α-Lactalbumin and Casein - in Normal and Neoplastic Mammary Tissue.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pran Chakrabartty	Visiting Fellow	LPP, NCI
OTHERS:	Pradman K. Qasba	Visiting Scientist	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	2/3	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To measure the levels of mRNA for mammary specific proteins - casein and α-Lactalbumin - in prelactating, lactating and neoplastic mammary cells. The steps, factors and influence of hormones involved in the synthesis and processing of these specific mRNA will be sought. Comparison will be made between normal and neoplastic tissue in relation to these regulatory steps.

Methods:
(1) Isolation of mRNA on poly U-Sepharase column and further purifications on gradients.
(2) In vitro protein biosynthesizing system derived from wheat germ which is dependent on exogeneous mRNA. The synthesis of specific proteins is measured by immunoprecipitation method.

Major findings:
(1) mRNA was isolated and purified from 5-7 days lactating rat mammary gland. This mRNA is efficiently translated in the wheat germ system. At present we are analysing the in vitro synthesized proteins for rat casein and rat αLA by immuno precipitating the proteins with their antibodies.
(2) Isolated nuclei from rat lactating mammary gland are able to synthesize in vitro RNA. In a transcriptional-translational coupled system isolated nuclie are able to synthesize proteins in wheat germ system. The synthesis of these proteins in this system is dependent on the combination of nuclei and wheat germ extracts. The synthesis of specific mRNA for casein and αLA and its translation is being studied.

Proposed Course of Research:
(1) Purification of casein and αLA mRNA.
(2) Synthesis of casein and αLA DNA probe.
(3) The synthesis and processing of mRNA in the coupled system will be investigated.

Significance to Cancer Research:
(1) DNA probes for casein and αLA will give a very sensitive method of determining the levels of mRNA in mammary neoplastic tissue.
(2) By learning the differences between normal and neoplastic cells in the transcriptional process we may be able to understand better the sequences leading to neoplastic transformation.

Publications: Components of Rat Lactose Synthetase: Purification and Properties of Rat αLA. Qasba, P. K. and P. Chakrabartty. Submitted for Publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08220-01 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Adenylation of Basic Cellular Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter R. Stone	Visiting Fellow	LPP, NCI
OTHERS:	William R. Kidwell	Head, Cell Cycle Regulation Section	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: Modification of proteins by the covalent attachment of various small molecules such as phosphate, acetate, methyl groups, etc. occurs extensively in living organisms. These modifications serve the cell either as fine regulatory controls allowing rapid responses to external stimuli (hormones) without new enzymes protein synthesis or they are involved in regulation of protein synthesis either at the transcriptional or translational level. One such modification recently discovered in our laboratory is the adenylation of a small basic cellular protein. This project concerns the characterization of the adenylation reaction, including the identification of the modified protein and the physiological significance of the adenylation reaction in the regulation of cellular metabolism.

PHS-6040
(12-75)

Methods: The adenylation reaction in intact cells is followed by means of pulsing cells with ^{3}H-adenosine followed by selective extraction of the adenylated protein into cold perchloric acid. The modified protein is then precipitated by addition of cold TCA. Final resolution of the adenylated proteins from other contamination radioactivity is achieved by electrophoresis of the TCA precipitated material on acid-urea polyacrylamide gels. For following the adenylation reaction in vitro, subcellular fractions are incubated with [^{3}H-adenosine]-ATP.

Major Findings: During a pulse labeling of Hela cells with ^{3}H-adenosine, a small basic protein becomes modified by the attachment of 5'-AMP. The adenylated protein is extractable from cells by a standard technique utilized for partial purification of H1 histone; that is, it is soluble in cold 5% PCA and precipitable from PCA with cold 18% TCA. The adenylated protein is readily resolved from the H1 histone by polyacrylamide gel electrophoresis. From its mobility on the gels it has an approximate molecular weight of 10,000 daltons. The same protein can be labeled in vitro by incubation of cell lysates with ^{3}H-adenosine, 70% of the modified protein is recovered from the crude nuclear fraction of the cells and 30% in the cytoplasm. However, highly purified nuclei do not synthesize any of the adenylated species. These results indicate that the modification takes place in the cytoplasm with subsequent translocation to the nucleus or that the adenylating enzyme is nuclear but leaks out of nuclei during purification.

The 5'-AMP-protein complex is very unstable at neutral pH. Pulse-chase experiments indicate a half-life of the order of a few minutes. The reaction is unusual in that it proceeds to completion in 10 seconds at 0 or 25°C in the lysates. Adenylation is maintained at a steady-state level until the substrate ATP is consumed and then the amount of adenylated protein disappears rapidly. Such a kinetic behavior is consistent with a catalytic reaction with a high turnover and a low K_M for ATP. ADP-Ribose, c-AMP, 2', 3'-AMP and adenosine have no effect on the adenylation reaction while 5'-ADP and 5'-AMP are inhibitors. The reaction does not appear to be a simple ATPase since it is not sensitive to fluoride ion or Oubain and the product is an adenylated protein. That the adenyl group is associated with a basic protein was shown by the fact that digestion with protease dramatically increased the electrophoretic modility of the radioactivity on the gels. From the amount of ^{3}H-ATP incorporated and assuming each acceptor protein molecule is joined with one adenyl group we can calculate that there is 0.03 µg of acceptor protein per $3x10^7$ cells or about $6x10^4$ acceptor protein molecules per cell (a lower limit estimate since the percent recovery is uncertain). Pulse labeling of the complex in whole cells with ^{3}H-adenosine indicated about equal amounts of the product was formed throughout S, G_2 and M phases of the cell cycle.

Significance for Cancer Research: (NCP Objective #6, Approach #3). Understanding the basis control mechanisms of cellular metabolism is essential for the successful design of ways to reimpose growth regulation on cancer cells whose normal controls are lost in the neoplastic state.

Proposed Course of Research: A major effort will be undertaken to evaluate the changes in the adenylation reaction of cells in various growth stages. The reaction will be compared in lymphocytes in G_0 and as a function of time as these cells are induced to divide by mitogens. A similar comparison will be made in cells grown in the presence and absence of inhibitors of protein, RNA and DNA synthesis. Effort will be extended to isolate the subcellular fraction in which the reaction takes place and to isolate and characterize the adenylated protein.

Publications:

1. P. R. Stone, R. Bredehorst, M. Kittler, H. Lengyel and H. Hilz. (1976) Hoppe Seyler's Z. Phys. Chem. 357: 51-56.

2. P. R. Stone and H. Hilz. (1975) Febs. Lett. 57: 209-212.

3. H. Hilz and P. R. Stone. (1975) Reviews of Biochemistry, Physiology and Pharmacology 76: In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08221-01 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Poly ADP-Ribosylation of Basic Nuclear Proteins in Growing and Regressing Mammary Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wishard S. Lorimer, III	Research Associate	LPP, NCI
OTHER:	William R. Kidwell	Head, Cell Cycle Regulation Section	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: Poly ADP-ribosylation of basic chromosomal proteins has been reported to occur both in vivo and in vitro. Functionally, this modification may present a mechanism for disrupting the electrostatic attraction between positively charged basic proteins and negatively charged chromosomal DNA. The reaction is thus likely to be involved in altering the degree of chromatin condensation during such processes as DNA and RNA synthesis, DNA repair or in preparation for mitosis. The objective of this project is to define in detail the changes in the poly ADP-ribosylation of nuclear proteins in actively growing mammary tumors and to quantitate the changes in this process when tumor regression is induced.

Methods: Two rat mammary tumor systems were evaluated in the study. One was a primary tumor induced by the carcinogen, 7,12-dimethylbenzanthracene. Regression of this tumor was affected by oophorectomy. The second tumor model was the transplantable tumor, MTW9, whose growth is dependent on peptide hormones released from a MTW5 pituitary tumor present in the same host. Regression of the MTW9 tumor was induced by surgical excision of the MTW5 tumor. Poly (ADP-Ribose) synthetic capacity in the tumors was evaluated by assessing the extent of incorporation of ^{3}H-NAD into the polymer in nuclei purified from the mammary tumors. Poly ADP-ribosylated basic proteins were selectively extracted from the nuclei by virtue of their differential solubility in cold PCA. These proteins were further characterized by electrophoresis on Acid-urea or urea-SDS polyacrylamide gels.

Major Findings: In human mammary cells (MCF-7) in culture we observed that poly (ADP-ribose) polymerase activity changed during the growth cycle. Polymerase activity was high in log phase cells and progressively declined as cells moved into a stationary, non-dividing stage. By late stationary phase, the level of polymerase dropped to 1/5 of that of log phase cells. A similar decline in polymerase activity was evident in tumors following removal of the source of growth-promoting hormones. By 24 hours after initiation of regression, poly (ADP-ribose) polymerase activity was reduced to 20-60% of the levels of control, growing tumors. After a variable lag period (3-7 days), polymerase activity increased to values equal to or exceeding those of controls. By analogy to the observations on MCF-7 cells, we can infer that during early regression, there is a selective enrichment in the population of tumor cells which are in a non-dividing state. Increased polymerase which occurs later in regression may reflect an activation of the polymerase or indicate a tissue enrichment in tumor cells whose growth is independent of steroid or peptide hormones; that is, an increase in the percent of cells which are dividing.

Characterization of the basic proteins which are poly ADP-ribosylated. The histone, H1 is reported to be the major nuclear protein to which poly-ADP-ribose) is joined *in vivo* and *in vitro*. Following incubation of rat mammary tumor nuclei with ^{3}H-NAD to label the polymer, the H1 protein was extracted and electrophoresed on acid-urea or SDS-urea gels. 90% of the protein moved on the gels as a single band and 70-90% of the radioactivity labeled poly (ADP-ribose) co-electrophoresed with this protein band. However, when the extracted material was electrophoresed on larger gels, the radioactivity could be resolved from the H1 histone band. This observation suggested that some protein other than H1 was in fact joined to poly (ADP-ribose). Consistent with this hypothesis was the demonstration that in human mammary cells (HBL100), no radioactive polymer is associated with the H1 histone. Rather, a new protein band in addition to H1 was detected on the gels. Labeled polymer was associated with the new protein peak. When poly ADP-ribose synthesis was limited either by omitting the substrate NAD or by including an inhibitor of poly (ADP-ribose) polymerase in the assay, the new protein species was absent from the PCA extract. These results indicate that a unique

basic protein is strongly associated with chromatin. Following covalent attachment of poly (ADP-ribose), the affinity of this protein is reduced and the protein-poly (ADP-ribose) moiety becomes extractable with PCA.

Significance for Cancer Research: (NCP Objective #6, Approach #3). The demonstration that poly (ADP-ribose) synthesis capacity is high in dividing tumor cells and decreases during early regression is evidence that polymer synthesis is necessary for cell growth. Additional experiments with inhibitors of poly (ADP-ribose) polymerase in tissue cultured cells offered more direct proof that this is the case. Should such inhibitors also be affective in intact animals, we will have uncovered a new target for the chemotherapeutic control of tumor cell proliferation.

Proposed Course of Research: Parallel studies will be performed on mammary tumors and mammary cell lines propagated in tissue culture. We will evaluate the effects of inhibitors of poly (ADP-ribose) synthesis on cell killing and evaluate anti-estrogens on regression. Correlation will be made between the potency of the inhibitors in limiting cell growth and their K_I on poly (ADP-ribose) synthesis. A major effort will be toward further characterizing the poly ADP-ribosylated nuclear proteins and the effect of this modification on chromatin structure.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08222-01 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Interrelationship of Cyclic Nucleotide and Steroid Hormone in Growth Control.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yoon S. Cho-Chung	Research Chemist	LPP, NCI
OTHERS:	Jeffrey S. Bodwin	Surgeon	LPP, NCI
	Timothy Clair	Chemist	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/4	1/2	3/4

SUMMARY OF WORK (200 words or less - underline keywords)

The early biochemical events occurring in the regression of DMBA tumors either due to steroid hormone withdrawal or due to dibutyryl cAMP (DBcAMP) injection to host animal, suggested the possible inter-relationship between cyclic nucleotide and steroid hormone in growth control. This investigation is focused on the relationship between cAMP-binding protein and estrogen receptor during growth and regression of DMBA tumors.

PHS-6040
(12-75)

Methods:
i) Tumors: Primary 7, 12-DMBA induced mammary carcinoma; regression is obtained by castration.
ii) cAMP-binding assay: the competition assay of Gilman was performed at 23° for 3 hr. ([^{3}H] cAMP binding to proteins includes not only free sites but also endogenously bound sites by the exchange).
iii) Adenylate cyclase assay: Activity of tumor homogenate was tested by the method of Krishna et al ± NaF utilizing AMP-PNP (ATP analogue) as the substrate.
iv) cAMP-phosphodiesterase assay: used the method of Thompson and Appleman.
v) estrogen binding assay: used dextran coated charcoal method at 23° for 3 hr. ([^{3}H] estradiol-17β binding to proteins includes not only free sites but also endogenously bound sites by the exchange).

Major Findings: A reciprocal relationship was found between estrogen binding capacity and cAMP binding activity in DMBA tumors during their growth and regression due to ovariectomy. Cytosol and nuclei of growing tumors possessed significant amount of specific estrogen binding components. During tumor regression the binding components of nuclei decreased; 3 days after ovariectomy, the binding activity of nuclei was reduced to about 50% of that of growing tumors. This decrease in nuclear estrogen binding activity was accompanied by the increase of cytosol binding activity about 2 fold. cAMP-binding by cytosol and nuclei of growing tumor was low. As early as 5 hr. after ovariectomy, however, cAMP binding by cytosol increased 5 fold; after 1 day of ovariectomy, the binding by nuclei started to increase. After 3 days of ovariectomy, the nuclei-associated cAMP binding was 10 fold of that of growing tumor, with concomitant decrease of cytosol cAMP binding. After 6 days of ovariectomy, the cytosol cAMP binding was almost as low as that of growing tumor, whereas the nuclei-associated binding remained at the peak activity. 3-5 fold increase in the activities of adenylate cyclase and cAMP-phosphodiesterase was also found in tumors after 5 hr. of ovariectomy. Injection of estradiol-17β into the host produced regrowth of the tumors and reversed the changes in binding capacity for estrogen and cAMP produced during regression. These results with DMBA tumors suggest a possible interaction at the nuclear level between estrogen receptors and cAMP-binding proteins.

Significance to Cancer Research: (NCP Objective #6, Approach #3)
These studies contribute to the understanding of the mechanism of hormone-dependent tumor regression. Studies on the interaction of estrogen receptor and cAMP-binding protein at nuclear level would clarify the mechanism by which steroid hormone interplay in abnormal growth, thus would contribute to the better prevention and treatment of abnormal hormone-dependent growth.

Proposed Course of Research:
i) Assess whether nuclear accumulation of estrogen receptor is competed by cytosol cAMP-protein complex, and whether nuclei-association of cAMP-binding protein can be inhibited by cytosol estrogen-receptor complex.
ii) Assess whether nuclear acceptor sites for cAMP-binding protein is also located in the acidic proteins of chromatin like estrogen-receptor.
iii) Assess whether a modification of chromatin proteins or DNA would inhibit

or activate estrogen or cAMP binding to nuclei.
iv) Assess whether specific mRNA would be produced by nuclei-association of estrogen receptor or cAMP-binding protein and whether the mRNA produced would be distinctive.
v) Assess whether specific enzyme protein synthesis would increase by the nuclei-association of these binding proteins.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08223-01 LPP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Estrogen Binding Studies in Ovarian Dependent and Independent Rat Mammary Carcinoma.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jeffrey S. Bodwin	Surgeon	LPP, NCI
OTHERS:	Yoon S. Cho-Chung	Research Chemist	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: To study the specific biochemical event of estrogen binding in relation to ovarian dependency of tumor growth. Also to assess quantitative or qualitative dofferences of estrogen binding proteins in ovarian dependent and independent tumors.

Methods:
1) Isolation of tumor cytosol and nuclei by differential centrifugation.
2) Sucrose density gradient sedimentation.
3) Estrogen binding measured by dextran coated charcoal method.
4) In vivo radionuclide injection to determine cytosol and nuclear binding of estradiol.

Major Findings:
1) DMBA rat mammary carcinomas possess specific estrogen binding activity with Kd λ 10^{-10} M.
2) Free sites only can be measured by DCC assay at 0° while total sites can be measured by DCC assay at 23° for 3 hrs.
3) Approximately 10% of DMBA carcinomas grow despite ovariectomy (ovarian independent) and possess significant amount of estrogen binding components.
4) Pre incubation of tumor cytosol at 37° C for 15 mins. of ovarian independent tumors revealed 40-60% retention of binding activity of unheated controls, whereas ovarian dependent tumors retained 10-40% of binding activity of unheated controls. It appears as if ovarian independent tumors possess more heat stable binding proteins than ovarian dependent tumors.

Significance to Cancer Research: (NCP Objective #6, Approach #3)
Characterization of estrogen binding of cytosol and nuclei of rat mammary carcinoma during growth and regression will serve as the basis for investigating the functional significance of hormone binding in growth control.

Course of Research: To study the qualitative and quantitative (i.e. heat stability) aspects of estrogen binding in ovarian dependent and independent carcinomas. To determine the functional role of hormone binding in the cytosol and nucleus of growing and regressing carcinoma.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08224-01 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Instability of Cytosol Proteins During Regression of Hormone Dependent Mammary Adenocarcinoma MTW9.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Maurice Rouleau	Visiting Fellow	LPP, NCI
OTHERS:	Pietro M. Gullino	Chief, Laboratory of Pathophysiology	LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To assess whether the regression of MTW9 can be explained in terms of instability of proteins produced by hormone deprivation.

Methods: Double-isotope labeling of tumor proteins in vivo. Proteolysis of cytosol proteins by exogenous proteases. Polyacrylamide gel electrophoresis and isoelectric focusing.

Major Findings:
1) The cytosol proteins isolated from a regressing MTW9 are more easily digested by a variety of exogenous proteases (trypsin, chymotrypsin, subtilisin BPN') than the proteins from a growing tumor.
2) This difference in susceptibility can neither be explained by the presence of an endogenous inhibitor or activator of proteases nor by the presence of an endogenous protease destroying the added enzyme.
3) The difference in susceptibility to proteolysis disappears after heating the protein solutions for 5 min. at 100°C or after protein precipitation by 80% ammonium sulfate.

The results of these experiments show that tumor regression after hormonal deprivation of the host is dependent on a labilization of cellular proteins as an initial event. The increment of lysosomal enzyme activity, usually described as the major event during regression, is in fact secondary to this labilization. The conditions under which the labilization of cellular proteins occur are being investigated.

Significance to Cancer Research: (NCP Objective #6, Approach #3)
It aims at an understanding of the biochemical control mechanisms by which a hormone dependent tissue regresses after hormone deprivation. Such an understanding might improve possibilities for rational therapy in hormone dependent human mammary tumors.

Proposed Course of Research: Characterization of the factor responsible for the difference in susceptibility to proteolysis and which can be eliminated by ammonium sulfate precipitation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08225 LPP

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure and Function of Biological Membranes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. Pinto da Silva, Head Membrane Biology Section LPP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathophysiology, DCBD

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the structure and function of biological membranes. The topics of present interest are:
1) comparative morphology of the plasma membranes from mammary tissue, normal and cancerous;
2) axolemma/Schwann cell relationships;
3) structural differentiations in myelin and Schwann cell membranes;
4) cell wall formation in zoospores from Phytophthora palmivora and mechanisms for membrane mediated release of cell wall precursor components;
5) vizualization of macromolecules on cell surfaces by scanning electron microscopy.

PHS-6040
(12-75)

LABORATORY OF THEORETICAL BIOLOGY

SUMMARY

July 1, 1975 through June 30, 1976

Summary Report

The activities of the Laboratory of Theoretical Biology (LTB) fall into several areas: the biophysics of membranes, immunology, molecular configurations, kinetics of metabolic systems and mathematical and computational methodology and theory related to modeling. Most of the work is theoretical, but some experimental work is also carried out in the laboratory. Much of the theoretical work and modeling activities are done in collaboration with experimental groups at NIH and elsewhere.

The work on membranes includes several areas of interest, mostly related to lipid bilayer systems (Blumenthal, Weinstein). The study of antibody-dependent lymphocyte-mediated cytotoxicity has been extended to use of the lipid antigen DNP-caproyl phosphatidyl ethanolamine. The lymphocyte-induced conductance changes are found to be highly sensitive to antigen concentration. Measurements of diffusion potentials in a salt gradient indicate that conductance pathways show no selectivity for Na^+, K^+, tetraethylammonium, Cl^-, or NO_3^-. The large increase in conductance is not due to a Ca^{++}-pathway.

All lymphocytes in a given incubation incorporate dye from vesicles (but not from free solution). Three components of fluorescence uptake are observed: (i) passive, (ii) active (i.e., dependent on metabolic activity), and (iii) adsorption of vesicles to the plasma membrane. Relief of self-quenching indicates escape of the dye from vesicles within the cell. Use of the fluorescence-activated cell sorter has permitted investigation of a variety of issues related to the phenomenology and mechanism of interaction.

A relationship is found between ionophoric activity in bilayers and mitogenic activity in mouse B-lymphocytes in the case of four high molecular weight proteins: excitability inducing material (EIM), keyhole limpet hemocyanin, polymerized flagellin and catalase. EIM is shown to be endotoxin-free by limlus lysate clotting assay and by its non-pyrogenicity in rabbits. Low molecular weight ionophores are toxic to the cells, and mitogenicity was not noted.

Experiments on bilayer conductance induced by purified dopamine-β-hydroxylase (DBH) have been continued using a different source and method of preparation of DBH. Consistent "channel-like" conductance changes are observed when DBH is added on both sides of the bilayer in the presence of 3-9 mM dopamine and 4 mM $CaCl_2$. The conductance increases as the square of the DBH concentration, shows no ion selectivity in the presence of KCl, and gives a symmetrical current-voltage curve.

The development of mathematical models for the interpretation of the dynamics of immune response systems was initiated this year when Dr. Charles DeLisi joined our group. Several theoretical problems are being investigated: mechanisms of ligand binding to surface receptors, the cross linking of receptors, plaque formations, and activation. The binding of multifunctional ligands to a surface with a dense homogeneous array of sites was also modeled and the results applied to the analysis of the phage inhibition assay. For immunoglobulin G the model consists of a two-step process: a bimolecular reaction followed by intramolecular bonding. It was possible to estimate the forward rate constant for the second step (a new number) from available data. A simple pilot model was also proposed for the interaction of tumor cells with killer lymphocytes. A careful theoretical investigation of this model (Rescigno) showed that despite its simplicity it can account for a number of interesting features observed experimentally. For example, the behavior is robust and includes the possibility of tumor-host coexistence at sufficiently low tumor levels; it permits oscillatory behavior; under special conditions tumor growth may be assisted by an increase of lymphocyte concentration. Sharper focusing on the model to include additional variables and the introduction of constraints based on available data will follow in an effort to obtain a better understanding of this important process.

The studies of conformation kinetics in polypeptides (Jernigan, Szu) have been extended to DNA. The aim is to understand the mechanisms and pathways of protein folding and denaturation and renaturation kinetics. Effects of perturbation direction and size were calculated. Strong dependences were predicted by the theory - in reasonable agreement with available experimental data.

The configurational patterns of macromolecules composed of a linear array of small molecules, as characterized by the distribution of distances between the small molecules, was investigated using Monte Carlo techniques. Asymetric, jagged distributions were observed. Analytical means for representing these will be investigated. These distributions relate to observed properties (optical, sedimentation, etc.) of the macromolecules in dilute solutions.

The modeling of metabolic systems (Berman) especially endocrine systems, is continuing. The model previously developed for glucose-insulin regulation in normals has been applied to obese and diabetic individuals, and for each subject a pattern of metabolism has been established which shows sensitivities of glucose production, glucose utilization and insulin secretion (E.A. McGuire). The glucose-insulin model is now being further developed to identify primary sites of defect that may explain the observed kinetic patterns in each subject. New features, extensions and refinements have also been added to the lipoprotein model, especially with regards to the apoproteins, triglycerides and cholesterol. Analysis of glycerol incorporation into VLDL (Zech) supports an earlier model of FFA incorporation into VLDL in which it is shown that at least two incorporation pathways are present - a fast one and a slow one. The glycerol studies also support the previously proposed step-wise delipidation process for VLDL. Modeling of cholesterol metabolism has also been initiated

in collaboration with the Veterans Administration group in Richmond, VA., (Schwartz, Swell). Cholesterol provides another moiety through which the lipoprotein particles may be viewed. Studies are also in progress on HDL apoproteins A-I and A-II (Schaefer) which show that the two apoproteins, though degraded together, are not synthesized in a fixed ratio to each other. An interesting seesaw degradation process has also been observed between vascular and extravascular degradation sites. Studies from endogenously labeled lipoproteins (Malmendier) have also validated much of the VLDL, IDL and LDL models previously developed and added new information on synthesis and the recycling of hydrolysis products.

Transport mechanisms between fetus, mother and amniotic fluid are being further investigated as a follow up of the model developed for thyroxine, triiodothyroxine and iodide (R. McGuire). The transport of water, creatinine, metabolites and other hormones are being studied in order to identify modes and sites of transport.

The moded for MTX kinetics developed earlier by Leme et al. has been applied to new studies carried out in the NCI Cancer Center in Baltimore, MD. The model was validated in part and in part modified to fit the new data. The old model was based on tracer data whereas the new one on data derived using immunoassay techniques (Reich).

The theory of stability, oscillations and pattern formations in biological systems are of continued interest to several members of the group. These have been applied to the study of regenerative processes (Trabert) and to tumor immune responses (DeLisi, Rescigno).

Kinetic theories dealing with chemical reactions using a stoichiometry oriented approach have been worked out. These permit a more formal approach to setting up the problem and contain new algorithms for their numerical solution (Park).

The SAAM computer program, used extensively as a modeling tool, has been further extended and tested to provide new features and more efficient computational techniques (Weiss, Chu). The new system is now identified as SAAM27.

In addition to the projects outlined above, members of the Laboratory have collaborated with a number of investigators on special models and provided consultations on mathematical, computational and modeling problems (Millar, Weiss, Goebel, McGuire, Zech).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 08300-04 LTB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

SAAM Computer Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Berman, Ph.D.	Acting Chief	LTB NCI
OTHERS:	M. F. Weiss	Mathematician	LTB NCI
	R. McGuire, Ph.D.	Staff Fellow	LTB NCI
	S. Chu, Ph.D.	Mathematician	LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Theoretical Biology

SECTION
Mathematical Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Continuing development of a computer system (SAAM) for the simulation, analysis and modeling of bio-kinetic systems. Further development of a conversational mode of operation, increased versatility of applications and automation are planned.

Project Description

Objectives: To develop a general purpose computer program for modeling biokinetic systems that may readily be used by investigators not sophisticated in mathematics or programming. Initiated in 1959, the program continues to be expanded and revised as new features are added.

Major Findings: New features were added to the program to permit more efficient computations of functions and differential difference equations (Weiss, Chu). Conversational SAAM was installed on the UNIVAC 1108 computer at the NBS making it directly compatible with the most updated versions of SAAM (McGuire). The SAAM program was also converted to operate under the CDC 7600 operating system at the Lawrence Berkeley Labs.

Significance to Biomedical Research and the Program of the Institute: The SAAM computer program has been a most essential tool in the modeling of various systems described elsewhere in this Laboratory report. Its wide use in other centers in the United States and elsewhere is further evidence of its value in biomedical research. It is consistent with Objective 4, Approach 4 of the National Cancer Plan.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08301-04 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Modeling Theory and Applications

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Berman, Ph.D.	Acting Chief	LTB NCI
OTHERS:	E.A. McGuire, Ph.D.	Staff Fellow	GRC NIA
	S. Reich, M.D.	Clinical Associate	BCRC NCI
	P. VanEerdewegh, Ph.D.	Visiting Fellow	LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of various kinetic and metabolic systems by the use of mathematical models is to be carried out in collaboration with other investigators. Specifically, the metabolism of glucose and insulin in man. These studies will serve to test and develop the SAAM computer program and modeling theory.

PHS-6040
(12-75)

Project Description:

Objectives: Analysis of data on metabolic systems, and further development of modeling theory and techniques through the applications of the SAAM computer program.

Major Findings: Studies in the kinetics of glucose and insulin have been carried out in normal, obese, diabetic and aged populations. Measures of sensitivity to glucose utilization to insulin, relative magnitude of insulin-dependent glucose metabolic pathways, rate of glucose production and insulin secretion at basal glucose levels have been determined for each patient. A model incorporating these patterns of changes is being formulated (McGuire).

The model for MTX kinetics developed earlier by Leme et al. has been applied to new studies carried out in the NCI Cancer Center in Baltimore. The model was validated in part and in part modified to fit the new data. The old model was based on tracer data whereas the new one on data derived using immunoassay techniques (Reich).

A measure has been proposed for the quantification of systematic deviations in data fitted to a function. This measure is now being tested (Van Eerdewegh).

Significance to Biomedical Research and the Program of the Institute: The methodology of modeling is most essential for studying the behavior of complex systems for both normal and abnormal states.

Proposed Course: This is a continuing process for the development of theory for and the application of modeling techniques.

Publications:

Leme, P.R., Creaven, P.J., Allen, L.M., Berman, M.: Kinetic model for the disposition and metabolism of moderate and high-dose methotrexate in man. Cancer Chemotherapy Reports, I. 59: 811-817, 1975.

Loevinger, R., Berman, M.: A schema for absorbed-dose calculations for biologically-distributed radionuclides. NM/MIRD, Pamphlet 1, Revised. New York, Society of Nuclear Medicine, Inc., 1976, pp. 1-10.

Froehlich, J.P., Albers, R.W., Koval, G.J., Goebel, R. and Berman, M.: Evidence for a new intermediate state in the mechanism of $(Na^+ + K^+)$-adenosine triphosphatase. The Journal of Biological Chemistry 251: 2186-2188, 1976.

Berman, M.: Kinetic models for absorbed dose calculations. NM/MIRD. New York, Society of Nuclear Medicine, Inc., 1976. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08302-04 LTB

PERIOD COVERED
July 1, 1975 through February 29, 1976

TITLE OF PROJECT (80 characters or less)
Computational Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. C. Chu, Ph.D. Mathematician LTB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Theoretical Biology

SECTION
Mathematical Biological Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

SUMMARY OF WORK (200 words or less - underline keywords)

The continuing goal in the automatic solution of systems of ordinary differential equations, as they arise in biological models, is an efficient computational algorithm handling both stiff and non-stiff systems.

Efficient and reliable methods for obtaining the asymptotic values of solutions of non-linear systems require improvements in the current non-linear algebraic techniques.

Project Description:

Objectives: To evaluate, develop and implement mathematical techniques used in the modeling and simulation of biological systems. This entails the development of efficient numerical procedures and their computational implementation.

Methods Employed: The primary mathematical tools needed to evaluate or develop an algorithm are from the theories of differential equations, numerical analysis, and linear algebra. Computer programming provides the means for implementation.

Major Findings: The numerical procedures and algorithms used in the solution of initial value problems for general systems of ordinary differential equations have been improved with regard to accuracy and efficiency.

A major effort was devoted to the consideration of hybrid dynamical systems; that is, systems which have the property that some of whose components are described by continuous dynamics and some by discrete dynamics. The continuous dynamics are those pertaining to differential equations, while a discrete component has subcomponents each of which is governed by discrete dynamical laws. An example of the latter is a discrete delay. The SAAM program already permits the delay feature. This effort is aimed at achieving simultaneously: a) greater generality, that is, larger classes of discrete components, and b) greater computational efficiency.

In conjunction with Mrs. M.F. Weiss, the above are being implemented and incorporated into the SAAM program for routine usage.

Significance to Biomedical Research and the Program of the Institute: Sound computational techniques facilitate and permit the mathematical analysis of complex biological systems.

Proposed Course: The analysis of the various mathematical aspects of biological modeling will be continued. An effort will be made to broaden the applicability of existing algorithms to more general systems as well as making them more computationally efficient.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08303-04 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Interactions of Ionophores and Lymphocytes with Lipid Bilayer Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Blumenthal, Ph.D.	Visiting Scientist	LTB	NCI
OTHER:	J. N. Weinstein, M.D., Ph.D.	Expert	LTB	NCI

COOPERATING UNITS (if any)

P. Henkart, Ph.D.	IB, NCI, NIH
D. L. Rosenstreich, M.D.	LMI, NCI, NIH
M. S. Kafka, Ph.D.	APB, NIMH

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

SUMMARY OF WORK (200 words or less - underline keywords)

A. Development of the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell membranes, and study of the physical mechanisms for ion transport. The bilayer membranes are formed from natural membrane extracts, oxidized cholesterol or pure lipids in an aperture between two electrolyte solutions. The electrical properties of the membranes are measured before and after application of an activating factor such as EIM (a bacterial protein which induces excitability), hemocyanin or dopamine-β-hydroxylase. The first phase of lymphocyte mediated lysis of target cells is studied by observing conductance changes induced by lymphocytes in an antibody coated antigenic lipid bilayer membrane.

B. Development of fluorescent dye-containing lipid bilayer vesicles as an assay for vesicle-cell fusion. The vesicles are incubated with human peripheral blood lymphocytes, and the resulting fluorescence is observed by microscopy, by fluorometry and by use of the fluorescence-activated cell sorter.

Project Description:

Objectives: To study the physical mechanisms of ion transport in reconstituted membranes. To develop the lipid bilayer membrane as an assay for ionophoric materials isolated from natural cell membranes. To study the interaction of lymphocytes with lipid bilayer membranes as a model for lymphocyte-mediated lysis of target cells. To study the role played in immune processes by the mobility and distribution of cell surface antigens. To develop fluorescent techniques for studying interaction of lipid bilayer vesicles with lymphocytes, with the possibility of targetting drug-containing vesicles for specific cells. To study the relationship between ionophoric and mitogenic properties of lymphocyte-activating compounds.

Methods Employed: The bilayer membranes are formed from natural membrane extracts, oxidized cholesterol, or pure lipids in an aperture between two electrolyte solutions. The electrical properties of the membranes are measured before and after application of an activating factor. In the lymphocyte experiments DNP-phosphatidyl ethanolamine is incorporated into a horizontal lipid bilayer, the settling of the lymphocytes on the lipid bilayer is observed through a phase contrast microscope, and the electrical conductance is measured.

Single-bilayer vesicles containing 200 mM 6-carboxyfluorescein are formed by sonication from dioleoyl phosphatidyl choline (DOL) or dipalmitoyl phosphatidyl choline. Free dye is removed on a Sephadex G-25 column. The vesicles are incubated with human peripheral blood lymphocytes, and the resulting fluorescence is observed by microscopy, by fluorometry, and by use of the fluorescence-activated cell sorter.

Preparation of mitogens and determination of mitogenic activity is performed in the laboratory of Dr. David L. Rosenstreich (LMI/NIDR). Dopamine-β-hydroxylase is prepared from bovine adrenal medullae in the Laboratory of Dr. Walter M. Lovenberg (HE/NHLI).

Major Findings:

A) The study of antibody-dependent lymphocyte-mediated cytotoxicity has been extended to use of the lipid antigen DNP-caproyl phosphatidyl ethanolamine. The lymphocyte-induced conductance changes are found to be highly sensitive to antigen concentration. Measurements of diffusion potentials in a salt gradient indicate that conductance pathways show no selectivity for Na^+, K^+, tetraethylammonium, Cl^-, or NO_3^-. The large increase in conductance is not due to a Ca^{++}-pathway.

B) All lymphocytes in a given incubation incorporate dye from vesicles (but not from free solution). Three components of fluorescence uptake are observed: (i) passive, (ii) active (i.e., dependent on metabolic activity), and (iii) adsorption of vesicles to the plasma membrane. Relief of self-quenching indicates escape of the dye from vesicles within the cell. Use of the fluorescence-activated cell sorter has permitted investigation of a variety of

issues related to the phenomenology and mechanism of interaction.

C) A relationship is found between ionophoric activity in bilayers and mitogenic activity in mouse B-lymphocytes in the case of four high molecular weight proteins: Excitability inducing material (EIM), keyhole limpet hemocyanin, polymerized flagellin and catalase. EIM is shown to be endotoxin-free by limlus lysate clotting assay and by its non-pyrogenicity in rabbits. Low molecular weight ionophores are toxic to the cells, and mitogenicity was not noted.

D) Experiments on bilayer conductance induced by purified dopamine-β-hydroxylase (DBH) have been continued using a different source and method of preparation of DBH. Consistent "channel-like" conductance changes are observed when DBH is added on both sides of the bilayer in the presence of 3-9 mM dopamine and 4 mM $CaCl_2$. The conductance increases as the square of the DBH concentration, shows no ion selectivity in the presence of KCl, and gives a symmetrical current-voltage curve.

Significance to Biomedical Research and the Program of the Institute: Increasingly it appears that ion permeabilities play an important regulatory role in cell maintenance and division; the lipid bilayer has been proven a sensitive tool for study of the molecular properties of ion-conducting channels in membranes. Elucidation of the mechanism of cell-mediated cytotoxicity has an obvious significance for tumor immunology. The vesicle-lymphocyte experiments have a direct bearing upon attempts to develop drug-loaded vesicles targetted for specific tumor cells. This work supports Objectives 3 and 6 of the National Cancer Plan.

Proposed Course: Our studies of cytotoxicity have progressed to a consideration of the role played in the immune processes by cell surface antigen mobility and distribution. Collaboration has been established with a group at Cornell University to use fluorescence photobleaching for that purpose. A small spot on the fluorescence-labeled cell surface is photolytically bleached by brief exposure to an intense laser beam, and subsequent recovery of the fluorescence is monitored. Recovery occurs by replenishment of intact fluorescent chromaphore via lateral transport into the bleached spot. We are currently putting together a fluorescence photobleaching apparatus in our own laboratory in order to study a variety of immunologic phenomena including mitogen-induced cytotoxicity and T cell-mediated cytotoxicity.

We plan continued studies to define the parameters important in vesicle-cell interaction. The effects of temperature, fluidity of the lipid, charge on the lipid, added proteins, and nature of the included solute are to be studied. Specific targetting will be tried using the DNP-α-TNP system. The role of Ca^{++} in mitogenesis will be explored by the use of vesicles to deliver C^{++}-specific buffers into lymphocytes.

Publications:

Henkart, P. and Blumenthal, R.: Interaction of lymphocytes with lipid bilayer membranes: A model for lymphocyte-mediated lysis of target cells. Proc. Nat. Acad. Sci., USA. 72: 2789-2793, 1975.

Blumenthal, R.: The interaction of hemocyanin and lymphocytes with lipid bilayer membranes. Ann. N.Y. Acad. Sci. 264: 476-482, 1975.

Blumenthal, R. and Shamoo, A.E.: The lipid bilayer membrane as an assay for ion-conductance-inducing materials isolated from biological membranes. Ann. N.Y. Acad. Sci. 264: 483-485, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08306-04 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetic Modeling of Human Plasma Lipoprotein Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Berman, Ph.D.	Acting Chief	LTB NCI
OTHER :	R. Goebel, M.D.	Research Associate	LTB NCI
	A. Zech, M.D.	Research Associate	LTB NCI
	C.L. Malmendier, M.D.	University of Brussels, Belgium	
	C. Schwartz, M.D.	Veterans Administration, Richmond, VA	

COOPERATING UNITS (if any)

Drs. R. Levy and Schaefer, Molecular Disease Branch, NHLI, NIH
Drs. S. Grundy and Steinberg, Veterans Administration, San Diego, CA

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

Kinetic models of plasma lipoprotein metabolism are being constructed, based on data from several kinds of tracer studies. Using the models, information from many sources is being integrated to build a quantitative understanding of plasma lipoprotein physiology in health and in disease. The models are particularly useful for the vigorous testing of hypotheses and the design of future experiments.

Project Description

Objectives: To develop a qualitative and quantitative understanding of lipoprotein metabolism in man and to identify abnormalities through the modeling of lipoprotein kinetics. Because of the need for diverse, extensive data on a variety of patients, collaboration with several experimental groups was established.

Methods Employed: Mathematical modeling is the tool used for the integration and analysis of the data. This is performed with the help of the SAAM computer modeling program.

Major Findings: A model was developed for HDL apoA. The results of the analyses suggest that there are two sites of degradation for HDL apoproteins and that perturbations tend to affect these in a seesaw fashion (Goebel). The apoB and apoC models previously developed have been extended to include the studies of Malmendier in Brussels. Although new features are required for the model, these are still not finalized. The essential features for the previous model, however, have been further validated (Malmendier). A new series of studies have been analyzed on the conversion of glycerol to VLDL. It was shown that glycerol required essentially the same kind of model as proposed earlier for FFA incorporation into VLDL. The glycerol model also supports the step-wise delipidation process for VLDL (Zech). A reexamination of the two compartment LDL model widely used in the literature is also in progress (Goebel).

An initial model has been derived for the pathways of cholesterol synthesis from a mevolonic acid precursor. The rates of incorporation of labeled precursor into bile acid, bile cholesterol and plasma cholesterol have been determined. The model also permits the calculation of rate of incorporation of plasma cholesterol into bile cholesterol and bile acids (Schwartz).

Significance to Biomedical Research and the Program of the Institute: Modeling becomes important in trying to isolate the abnormality responsible in hyperlipemics, given a very complicated system with complicated interactions. In the long run, it is hoped that by isolating the abnormality, treatment can be geared to the abnormal site identified. Clinically, this is relevant to atherosclerosis, cardiovascular disease, and other metabolic disorders.

Proposed Course: The results obtained with protein kinetics will be further integrated with experiments involving other lipoprotein moieties, such as the triglycerides. Some work on cholesterol has also been initiated.

Publications:

Phair, R.D., Hammond, M.G., Bowden, J.A., Fried, M., Fisher, W.R. and Berman, M.: A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. Federation Proceedings 34: 2263-2270, 1975.

Fried, M., Fisher, W.R., Hammond, M.D., Bowden, J.A., Berman, M. and Phair, R.D.: Computer model of lipoprotein metabolism in hyperlipoproteinemia. In Peter, H. (Ed.): Protides of the Biological Fluids 22nd Colloquium. N.Y. Pergamon Press, 1975, pp. 621-625.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08312-03 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Computer Applications: Programming and Data Processing

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: F. K. Millar, Ph.D. Computer Specialist LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

To develop software for data processing and other computer applications in the investigation of selected biomedical research problems.

PHS-6040
(12-75)

Project Description:

Objectives: To facilitate the investigation of biomedical research problems through the application of computer programming and computer data processing.

Methods Employed: Use is made of the PDP-11/20 computer of this laboratory, the National Bureau of Standards Univac 1108 computer, and the central NIH computer facility. The projects reported may be collaborative efforts with investigators in other units, as shown below.

Major Findings:

Computer Applications:

(1) Analysis of feeding patterns in rats (Dr. S. D. Morrison, C/LPHY, now C/LPP). Data, produced as rats feed from an eatometer, and collected on magnetic tape cassette, give, after appropriate corrections, direct measurement of feeding intensity which can then be used to calculate actual meal lengths and inter-meal intervals. Earlier approaches for the reduction, correction and analysis of these data by the PDP-11/20 computer are being adapted to an on-line Wang 2200 system.

(2) Size distributions of thyroid follicles, cells and cell components. (Dr. J. P. Herveg, C/LPHY). Two-dimensional distributions of cell size parameters (e.g., major and minor axes) have been analyzed by the method of Wicksell (Biometrika 17: 84, 1925) using the program of Baudhuin (Methods in Enzymology 32: 3, 1974) to calculate the actual distributions in three dimensions of these tissue components.

(3) Processing and plotting of data from liquid scintillation spectrophotometers; comparison of isoaccepting species of tRNA isolated from normal and malignant cells. (Dr. J. F. Mushinski, Dr. M. S. Marini, C/LCBGY).

Data collected on punched paper tape are reduced and analyzed on the PDP-11/20. Final results may be plotted directly on the on-line CalComp plotter but are also saved on magnetic tape for future use.

(4) A simple search-and-retrieval program has been written to operate on information files such as a bibliography or a name-and address list. The search progresses under direction of user-defined commands which specify the type of search, the items to be searched for and the format for print-out of the results.

(5) A general purpose plotting package has been developed and implemented for the CalComp plotter on-line to the PDP-11/20. A package of similar design is planned for local use of an EAI- 230 plotter connected in demand mode to the National Bureau of Standards Univac 1108 computer. These two systems for plotting will provide different advantages related to speed, availability and versatility.

Significance to Biomedical Research and the Program of the Institute: Computer programming applications contribute to the investigation of research problems by providing (1) accurate and efficient methods for handling laborious calculations of data and visualizing results, (2) the ability to store and retrieve large amounts of data, and (3) opportunities for testing mathematical models based on experimental results. This project most closely supports Objective 4, Approach 4 of the National Cancer Plan by providing methods of developing mathematical models of the cancerous process.

Proposed Course: Programs will continue to be developed and computational techniques to be applied in response to computer-related needs of the scientific staff.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08313-03 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Dynamical Models of Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. K. Trabert, M.D., Ph.D. Sr. Research Associate LTB NCI

OTHER: S. Kauffman, M.D., Dept. of Biochem. & Biophys., Univ. of PA.

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

A morphogen model representing the reaction and diffusion of several chemical compounds was investigated to see whether it showed the qualitative behavior seen in the development of Drosphila Melangaster and Amphibia. The formation of sequences of compartments during development of the imaginal wing disks of Prosphila was found to agree with experiment. Regenerative and duplicative phenomena were also investigated. Rules for the pattern of growth may also be deduced from the theory which will be compared to experiment. The results from this model will be used to suggest further experiments and will be compared with data derived from developmental phenomena for other imaginal disks and other species.

PHS-6040
(12-75)

Project Description

Objectives: To qualitatively understand the developmental phenomena that occur in maturing embryoes.

Methods Employed: Proposing dynamical morphogen models and testing them against experimental data.

Major Findings: The formation of compartments in the wing dish of Prosophila has been qualitatively explained from the properties of a reaction-diffusion dynamical system. A sequence of compartments can be deduced from the theory which agrees with the experimental observations. A rule for the pattern of growth of the wing dish of Drosophila can also be deduced from the theory which qualitatively fits the data.

Significance to Biomedical Research and the Program of the Institute: This project most closely supports Objective 4, Approach 4 of the National Cancer Plan. The variables that regulate normal development can be qualitatively understood using these dynamical systems. Abnormal development, as in the cancerous process may also be understood in these terms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08315-02 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Analysis of Oscillatory and Evolutionary Behavior in Dynamical Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. K. Trabert, M.D., Ph.D. Sr. Research Associate LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical models which simulate general biochemical kinetic reactions were studied by analytical means to determine stability and oscillatory properties. New work was begun on systems of differential equations which evolve in the course of time to behavior represented by other dynamical systems. The study of these evolutionary dynamical systems and their application will be continued.

PHS-6040
(12-75)

Project Description

Objectives: Mathematical models which simulate general biochemical kinetic reactions were studied by analytical means to determine regions of relative stability.

Methods Employed: Analytical and mathematical techniques were employed to derive theorems about oscillating and evolutionary systems.

Major Findings: A negative criterea for the existence of oscillations in dynamical systems was generalized to four variable dynamical systems. The method was also shown to be extendable to any even dimensional system. Differential equation systems which evolve in the course of time to different differential equations were also studied to understand their stability and oscillatory properties. The study of this type of dynamical systems which show evolutionary properties is a new mathematical field.

Significance to Biomedical Research and the Program of the Institute: This project most closely supports Objective 4, Approach 4 of the National Cancer Plan by developing mathematical models of the cancerous process. The study of evolutionary dynamical systems may play a role in the understanding of normal and abnormal development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08316-02 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetic Modeling of Thyroid Function in Man and Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Berman, Ph.D.	Acting Chief	LTB NCI
	R. McGuire, Ph.D	Staff Fellow	LTB NCI
OTHER:	B. Belshaw, D.V.M.	Cornell Medical Center, N.Y.	
	M. Hays, M.D.	V.A. Central Office, Washington D.C.	
	K. Burman, M.D.	Walter Reed Hospital, Washington, D.C.	
	L. Wartofsky, M.D.	Walter Reed Hospital, Washington, D.C.	
	J. Robbins, M.D.	Chief	CEB NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Kinetic models of thyroid hormone metabolism are being developed for man and other species using collaborative and literature data. The models are used to integrate data, to test hypotheses of normal and abnormal system functions and regulations, and to suggest future experimental approaches.

Project Description:

Objectives: To develop a quantitative understanding of thyroid hormone synthesis, distribution and catabolism in man and other animals; to study the changes occuring during normal development and during disease states; and to study the effects of pharmacologic agents in the thyroid system.

Methods Employed: Integration and analysis of collaborative studies and literature information within a comprehensive mathematical model. The model is developed using the SAAM computer modeling system.

Major Findings: T_4 to T_3 conversion in thyroidectomized humans has been modeled under varying levels of T_4 and T_3 administration. Fractional conversion of T_4 to T_3 did not change under changing T_4 and T_3 levels. Changes in model parameters have been quantified in populations of dogs on various iodine intakes. These model changes correlate well with observed morphological changes suggesting shifts in cell populations (Belshaw). The model for fetal metabolism and transport of T_4, T_3 and I^- has been further refined and supports the idea that T_3 is actively transported from the fetus to the mother. Some modeling of the kinetics of TBG in the rhesus monkey before and after the administration of estrogen has also been attempted and possible sites of action of the hormone on the metabolism of TBG have been suggested (Robbins).

Significance to Biomedical Research and the Program of the Institute: Modeling provides a means for detailed analysis of metabolic systems in normal and abnormal states.

Proposed Course: 1) Continued development of fetal system, 2) development of TRH and TSH models, 3) integration of TRH and TSH models into the thyroid model.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 08317-02 LTB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Theory of Conformational Transitions in Polypeptides and DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Jernigan, Ph.D.	Theoretical Physical Chemist	LTB	NCI
OTHER:	S. C. Szu, Ph.D.	Visiting Fellow	LTB	NCI

COOPERATING UNITS (if any)
None

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SECTION
Mathematical Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to elicit molecular details of conformational kinetics in polypeptides and DNA. An eventual aim is to understand the mechanisms and pathways of protein folding and of denaturation and renaturation kinetics. A mathematical model of the rates for passing between the various configurational states was previously formulated. Molecular states are determined by specification for each residue of a helical or random coil conformation. Averages are performed over all possible conformations. Calculated results have been compared with experiments on DNA. Effects of perturbation direction and size were calculated; strong dependences were observed, mostly in agreement with corresponding experiments. The effects of including a bimolecular reaction for the formation of the first DNA helix unit were investigated; for usual conditions this effect is small. Studies will be extended in order to attempt to account for observed molecular weight dependences of the relaxation behavior.

PHS-6040
(12-75)

Project Description

Objectives: To elicit molecular details of conformational kinetics in polypeptides and DNA. An eventual aim is to understand the mechanisms and pathways of protein folding and denaturation and renaturation kinetics.

Methods Employed: A mathematical model of the rate for passing between the various configurational states was previously formulated. Molecular states are determined by specification for each residue of a helical or random coil conformation. Averages are performed over all possible conformations.

Major Findings: The previous model, appropriate for polypeptides, has been extended to treat conformational relaxation in deoxyribonucleic acids. A major reason for this extension is the possibility of making direct comparisons with abundant data. Effects of perturbation direction and size were calculated; strong dependences were observed, mostly in agreement with corresponding experiments. The effects of including a bimolecular reaction for the formation of the first DNA helix unit were investigated; for usual conditions this effect is small.

Significance to Biomedical Research and the Program of the Institute: A basic understanding of denaturation and renaturation of DNA and of proteins is essential for understanding cellular processes on a molecular basis. Such comprehension is necessary before detailed effects of external chemical agents can be determined. This is pertinent to Objective 1, Approach 2 and Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: Calculations will be extended to attempt to account for observed molecular weight dependences of the relaxation behavior. The effects of specific sequences of residues should also be investigated; in particular, it would be interesting to know which sequences of units would lead to either a single average relaxation time or to separate relaxation times for distinct molecular regions.

Publications:

Jernigan, R. L. and Szu, S.C.: Polypeptide relaxation rates with configurationally dependent internal friction. J. Poly. Sci. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08318-02 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Molecular Mechanics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Jernigan, Ph.D.	Theoretical Physical Chemist	LTB	NCI
OTHER:	S. C. Szu, Ph.D.	Visiting Fellow	LTB	NCI

COOPERATING UNITS (if any)

G. Weiss, Ph.D. PSL, DCRT, NIH
S. Hsiao PSL, DCRT, NIH
R. J. Roe, Ph.D. Dept. of Chemistry, Univ. of Cincinnati, Cincinnati, Ohio

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to predict dilute solution behavior with detailed molecular models for internal macromolecular conformations. Experimental information on small molecules is combined with a one-dimensional configurational model to permit calculation of properties of large linear molecules. Each chemical bond is permitted to assume several rotational isomeric states of fixed geometry. Nearest neighbor dependent probabilities of these states are included. The theory is valid for linear molecules of any length.

Investigations of intramolecular distances by Monte Carlo methods revealed asymmetric, jagged distributions for finite chain molecules. Analytical means of representing the intrachain distance distributions will be investigated.

Project Description:

Objectives: The purpose is to predict dilute solution behavior with detailed molecular models for internal macromolecular conformations.

Methods Employed: Experimental information on small molecules is combined with a one-dimensional configurational model to permit calculation of properties of large linear molecules. Each chemical bond is permitted to assume several rotational isomeric states of fixed geometry. Nearest neighbor dependent probabilities of these states are included. The theory is valid for linear molecules of any length.

Major Findings: Investigations of intramolecular distances by Monte Carlo methods revealed asymmetric jagged distributions for finite linear chain molecules. These results directly affect most chain molecule properties.

The theory of the effects of internal conformations on dichroism was extended to account for some of these effects.

Significance to Biomedical Research and the Program of the Institute: These calculations permit prediction of a variety of physical properties for linear molecules of any given monomer sequence. Such calculations in advance of laboratory experiments can provide guidance to the experimental scientist. This is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: Analytical representations of the intrachain distance distributions will be investigated. Calculations of quantities to be measured by dichroism will be performed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 08319-02 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Population Genetic: X-Linked Transmission

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Van Eerdewegh	Visiting Fellow	LTB NCI
OTHER:	M. Van Eerdewegh, M.D.	Guest Worker (Grant: F.R.S.M., Belgium) -	APB NIMH
	E. Gershon, M.D.	Chief	APB NIMH

COOPERATING UNITS (if any)

None

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Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed an X-chromosome threshold model of genetic transmission. This model has been applied to data on manic-depressive illness, namely for testing the transmission of the illness (Unipolar form (UP) and Bipolar form (BP)) in families of Bipolar proband. The Bipolar form of the illness is compatible with an X-linked transmission whereas the UP forms seem to be more heterogeneous from a genetic point of view. However, we have identified in a particular study a UP form transmitted by the same X-locus responsible for the BP manic-depressive illness.

Project Description

Objectives: We have developed an X-chromosome threshold model of genetic transmission. When more than two phenotypes are possibly associated with the genetic expression of a single locus, this model, by the choice of the number of thresholds and/or their significance, allow us to test hypothesis about the genetic diathesis shared by those phenotypes.

Major Findings: The X-chromosome threshold model has been applied to data on manic-depressive illness, namely for testing the transmission of the illness (Unipolar form (UP) and Bipolar form (BP)) in families of Bipolar proband. The Bipolar form of the illness is compatible with an X-linked transmission whereas the UP forms seem to be more heterogeneous from a genetic point of view. However we have identified, in a particular study, a UP form transmitted by the same X-locus responsible for the BP manic-depressive illness.

Significance to Biomedical Research and the Program of the Institute: This kind of approach can be used for testing the hypothesis of a single gene (with complete or incomplete penetrance) factor in the transmission of qualitative or quantitative traits and also to predict the prevalence of a trait among the relatives of a proband.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08320-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Protein Conformational Theory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Jernigan, Ph.D.	Theoretical Physical Chemist	LTB	NCI
OTHER:	S. C. Szu, Ph.D.	Visiting Fellow	LTB	NCI

COOPERATING UNITS (if any)

T. T. Wu, M.D., Ph.D. Northwestern University
H. Bilofsky, Ph.D. Bolt, Beranek & Newman, Inc. Camb., MA
E. Kabat, Ph.D. NCI, NIH; Columbia Univ., Presbyterian Hospital N.Y.
S. Bourgeois, Ph.D. The Salk Institute for Biological Studies, CA

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Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Previously available statistical and theoretical methods for predicting protein secondary structures have been applied to several proteins. Improved predictions are obtained when there is agreement among the results obtained with several methods. This combination method was applied to a group of five immunoglobulins. Predictions compared favorably with their structures from X-ray experiments. Secondary structures in lac repressor were predicted; its absolute conformation is unknown.

Further applications of the combination method are planned for several other classes of proteins.

PHS-6040
(12-75)

Project Description:

Objectives: To predict protein conformations from their sequences and to determine the important residue-residue interactions.

Methods Employed: Previously available statistical and theoretical methods for predicting protein secondary structures have been applied to several proteins.

Also, statistical residue-residue distance distributions have been derived from available protein X-ray structures.

Major Findings: Improved predictions are obtained when there is agreement among the results obtained with several methods. This combination method was applied to a group of five immunoglobulins. Predictions compared favorably with their structures from X-ray experiments. Secondary structures in lac repressor were predicted; its absolute conformation is unknown.

Significance to Biomedical Research and the Program of the Institute: Development of a reliable method for predicting protein conformations from their sequences would yield understanding of numerous biochemical reactions and processes.

Proposed Course: A theory to utilize the residue-residue distance distributions for the prediction of protein conformations is proposed. We plan a further application of the combination secondary structure prediction method to other classes of molecules, such as neurotoxins and lipoproteins. Biochemical data on the behavior of known sequence mutants may be a useful adjunct in developing protein conformational theory. Additional methods for predictions of higher order structures in proteins will be considered.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08321-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetic Modeling of Maternal, Fetal and Amniotic Fluid Exchanges

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. McGuire, Ph.D.	Staff Fellow	LTB	NCI
	M. Berman, Ph.D.	Acting Chief	LTB	NCI

COOPERATING UNITS (if any)

None

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Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Kinetic models of maternal-fetal exchange of nutrients (iodide), metabolites (createnine), hormones (T_3, T_4) and drugs are being developed for man and other species using literature data. The models are used to integrate data, to test hypotheses of normal and abnormal system functions and regulations. The model will be useful for future designs of therapeutic regimens for pregnant women.

Project Description:

Objectives: To study the transport of substances between the mother, fetus and amniotic fluid in order to gain an understanding of the mechanisms of transport. This is an extension of previous work carried out on the transport of thyroid hormones (previous report No. Z01 CBD 08316-01 LTB).

Major Findings: A model for createnine exchange between maternal, fetal and amniotic fluid was developed to determine exchange values for this unmetabolized excretion product. In contrast to T_4, T_3 and I^-, a direct exchange between maternal and anmiotic fluid was observed. Preliminary water and urea models also exhibit these direct paths.

Significance to Biomedical Research and the Program of the Institute: A model provides a means for the prediction of the behavior of metabolic systems in normal and abnormal states. Knowledge of the fetal disposition of therapeutic agents or environmental toxicants administered to or consumed by the mother is necessary to understand possible effects of these on the fetus. Such knowledge could also aid in the design of effective delivery regimens of therapeutic agents to the fetus. Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: Continued development of transport models for substances for which data are available in the literature and that are of potential use in the elucidation of transport mechanism. Some of the substances to be studied include water, urea, alcohol and nicotene.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08322-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physical Chemistry of Lymphocyte Activation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. DeLisi, Ph.D. Theoretical Biologist (I.P.A.) LTB NCI

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this work so far has been to pursue the consequences of the idea that receptor cross linking is a necessary (though perhaps not sufficient) condition for cellular activation. The theory predicts that for antigen concentrations within a certain range, the single antigen receptor complexes which form initially will begin aggregating, slowly at first, and then abruptly coalesce into large "patches". If such coalescence is assumed to be a requirement for activation, then: 1) the cell will not respond to excessively high or low antigenic doses; 2) the affinity of cells stimulated increases with time after immunization; 3) the cell will not respond if the valence of the antigen is too low. The theory thus distinguishes binding reactions from triggering reactions and predicts affinity maturation. Moreover (3) also suggests a requirement for helper cells which would aggregate antigen and effectively increase its valence. There is abundant experimental evidence for (1) and (2) and some data consistent with (3).

The theory was also extended to analyze the relationship between intramolecular reaction and cross linking. The two processes are competitive and specific experiments were outlined which would utilize variations in receptor density, membrane viscosity and antigen valence to investigate the nature of the competition and the consequences for activation.

Project Description:

Objectives: To develop an understanding of the relationship between the thermodynamics and kinetics of ligand-cell bound receptor interactions, and the requirements for lymphocyte activation.

Methods Employed: Mathematical Modeling

Major Findings: The major goal of this work so far has been to pursue the consequences of the idea that receptor cross linking is a necessary (though perhaps not sufficient) condition for cellular activation. The theory predicts that for antigen concentrations within a certain range, the single antigen concentrations within a certain range, the single antigen receptor complexes which form initially will begin aggregating, slowly at first, and then abruptly coalesce into large "patches". If such coalescence is assumed to be a requirement for activation, then; 1) the cell will not respond to excessively high or low antigenic doses; 2) the affinity of cells stimulated increases with time after immunization; 3) the cell will not respond if the valence of the antigen is too low. The theory thus distinguishes binding reactions from triggering reactions and predicts affinity maturation. Moreover (3) also suggests a requirement for helper cells which would aggregate antigen and effectively increase its valence. There is abundant experimental evidence for (1) and (2) and some data consistent with (3).

The theory was also extended to analyze the relationship between intramolecular reaction (antigen bound to two sites on the same receptor) and cross linking (antigen bound by two sites on different receptors). The two processes are competitive and specific experiments were outlined which would utilize variations in receptor density, membrane viscosity and antigen valence to investigate the nature of the competition and the consequences for activation.

Proposed Course: The models developed so far parameterize the effects of other cells and a multitude of factors which are involved in immune recognition and control. The important control aspects of the response can never be adequately understood this way and future efforts will attempt to incorporate cell-cell interactions.

Significance to Biomedical Research and the Program of the Institute: Understanding the basis of immune recognition and control (including paralysis and unresponsiveness) would clearly be an important step in understanding conditions under which a host's immune system might be effective in eliminating or holding in check incipient tumors, and in providing a rational basis for immunotherapy. This project is a link between assay quantitation and immune surveillance models.

Publications:

DeLisi, C. and Perelson, A.: Kinetics of aggregation phenomona. I. Minimal models for patch formation of lymphocyte membranes. J. Theor. Biol. (In press).

DeLisi, C.: Antigen binding to B-cell immunoglobulin receptors (invited paper) to be published in Theoretical Immunology, G. I. Bell (ed.).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08323-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Quantitation of Immunochemical Assays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. DeLisi, Ph.D. Theoretical Biologist (I.P.A.) LTB NCI

COOPERATING UNITS (if any)

None

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SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

A kinetic model for binding multifunctional ligand to a surface with a dense homogeneous array of sites was developed and applied to the analysis of the phage inhibition assay, which exploits the loss of bacteriolytic capacity that occurs as a consequence of the phage antibody interaction. For immunoglobulin G, the interaction is a two step process; a bimolecular reaction followed by intramolecular bonding. It was possible to estimate the forward rate constant for the second step (a new number) from available data.

Some in vitro and in vivo implications of miltisite attachment have been developed as part of a broader collaborative effort with Dr. Henry Metzger, NIMADD, in which factors contributing to observed rate constants were analyzed.

The hemolytic plaque assay, which is used world wide for detecting and enumerating antibody secreting cells, was further analyzed in the presence of an electric field. Immunologists have not attempted electrophoresis experiments which use lymphocytes as an antibody source, but the analysis suggests they are feasible and should be of considerable importance.

Project Description

Objectives: To quantitate certain immunological assays and delineate conditions under which physical chemical information can be obtained.

Methods Employed: Mathematical Modeling

Major Findings: A kinetic model for binding multifunctional ligand to a surface with a dense homogeneous array of sites was developed and applied to the analysis of the phage inhibition assay, which exploits the loss of bacteriolytic capacity that occurs as a consequence of the phage antibody interaction. For immunoglobulin G, the interaction is a two step process; a bimolecular reaction followed by intramolecular bonding. It was possible to estimate the forward rate constant for the second step (a new number) from available data.

Some in vitro and in vivo implications of multisite attachment have been developed as part of a broader collaborative effort with Dr. Henry Metzger, NIMADD, in which factors contributing to observed rate constants were analyzed.

The hemolytic plaque assay, which is used world wide for detecting and enumerating antibody secreting cells, was further analyzed in the presence of an electric field. Immunologists have not attempted electrophoresis experiments which use lymphocytes as an antibody source, but the analysis suggests they are feasible and should be of considerable importance.

Significance to Biomedical Research and the Program of the Institute: In order to develop a complete understanding of the relationship between the immune system and tumors, physical chemical aspects of their interaction must be characterized. This project is working toward that goal - Object 4, Approach 4.

Proposed Course: Mathematical modeling has provided a sound foundation for several assays, especially the hemolytic plaque technique which can now be confidently used along with the theory to obtain biologically important information. Future efforts are expected to involve collaborative experimental work with Drs. Bruce Merchant (Bureau of Biologics) and John Inman (NIAID) to explore the possibility of extending the technique to obtain secretion rate characteristics. This possibility is a clear prediction of the theory; testing it will present no technical difficulties and if successful it will represent an important extension of the method.

Publications:

DeLisi, C.: Antigen-Antibody Interactions. Heidelberg, Springer Verlag, 1976, pp. 144, (in press).

DeLisi, C. and Metzger, H.: Some physical chemical aspects of receptor ligand interactions. In Cinader, B. (Ed.) Immunology of Receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CBD 08324-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mathematical Models of Immune Surveillance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. DeLisi, Ph.D.	Theoretical Biologist (I.P.A.)	LTB	NCI
OTHER:	A. Rescigno, Ph.D.	Mathematician (I.P.A.)	LTB	NCI
	A. K. Thakur, Ph.D.	Visiting Fellow	LTB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

A simplest possible model involving only tumor cells and killer lymphocytes was developed and extensively studied. It was found that even in this simple two variable system, the behavior is robust and includes the possibility of tumor-host coexistence at sufficiently low tumor levels, and oscillatory behavior.

The predicted results of immune suppression or stimulation are in general agreement with observation and intuitive expectations. In addition, however the theory predicts that there will be isolated instances under which an increase in lymphocyte concentration may assist tumor growth. This phenomonon is also observed experimentally and the theory provides a possible explanation (and so far as I know, the only one) which is logically consistent with immune surveillance.

The model in its most primitive form, however, does not allow de novo development.

It was shown that making the model slightly more realistic by introducing a population of precursor lymphocytes which must go through some maturation period before they are effective in destroying tumor cells, removes this difficulty.

Project Description

Objectives: To develop a qualitative understanding of the dynamics of the host-tumor cell interaction.

Methods Employed: Computer simulation, mathematical analysis.

Major Findings: A simplest possible model involving only tumor cells and killer lymphocytes was developed and extensively studied. It was found that even in the simple two variable system, the behavior is robust and includes the possibility of tumor-host coexistence at sufficiently low tumor levels, and oscillatory behavior.

The predicted results of immune suppression or stimulation are in general agreement with observation and intuitive expectations. In addition, however the theory predicts that there will be isolated instances under which an increase in lymphocyte concentration may assist tumor growth. This phenomonon is also observed experimentally and the theory provides a possible explanation (and so far as I know, the only one) which is logically consistent with immune surveillance.

The model in its most primitive form, however, does not allow de novo tumor development.

It was shown that making the model slightly more realistic by introducing a population of precursor lymphocytes which must go through some maturation period before they are effective in destroying tumor cells, removes this difficulty.

Proposed Course: The model will be increased gradually in complexity, assessing at each stage the effects of the new additions. More detailed comparisons with experimental data are also expected to be possible within the coming year.

Significance to Biomedical Research and the Program of the Institute: Understanding the dynamics of the tumor-cell immune system interaction may have important implications for immunotherapy. Mathematical modeling may make a significant contribution to developing this understanding.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 08325-01 LTB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Compartment Modeling of Complex Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Rescigno, Ph.D. Mathematician (I.P.A) LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Theoretical Biology

SECTION
Mathematical Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

To construct a model of a complex system involving a large number of compartments, some important parameters can be computed from the experimental data available from tracer experiments. From those parameters a model compatible with the data, but containing a minimum number of compartments is first constructed; then new methods are investigated to transform this model into another one with the same parameters but a different number of compartments.

PHS-6040
(12-75)

Project Description

Objectives: To construct in the most efficient way models of complex systems involving a large number of compartments.

Methods Employed: Operational calculus.

Major Findings: A number of parameters, called Weight, Transfer Time, and Dispersion, can be computed in a simple and economical way from the experimental data available from tracer experiments. From those parameters a model compatible with the data, but containing a minimum number of compartments, can be constructed.

Significance to Biomedical Research and the Program of the Institute: Modeling of complex systems is essential to the understanding of normal and pathological states. This project closely fits Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: The problem to be solved now is how to transform a model into another one with the same parameters but with a different number of compartments.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CBD 08326-01 LTB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Dynamical System Theory in Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Rescigno, Ph.D. Theoretical Biologist (I.P.A.) LTB NCI
K. K. Trabert, M.D., Ph.D. Sr. Research Associate LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Theoretical Biology

SECTION
Mathematical Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The non-linear differential equations describing the behavior of a population of molecules, or of cells, or of individuals, are studied in the context of systems theory. Necessary and sufficient conditions for the existence of enclosures are sought, and their biological importance investigated.

PHS-6040
(12-75)

Project Description

Objectives: To study the general properties of the non-linear differential equations describing the behavior of a population of molecules, or of cells, or of individuals, subject to different kinds of interaction.

Methods Employed: The primary mathematical tool used in dynamical system theory is the theory of autonomous non-linear differential equations.

Major Findings: The major results so far are sufficient conditions for the existence of enclosures in systems with any number of dimensions.

Significance to Biomedical Research and the Program of the Institute: The mathematical analysis is a prerequisite to the understanding of complex biological systems; this project is thus consistent with Objective 4, Approach 4 of the National Cancer Plan by developing new mathematical models of the cancerous process.

Proposed Course: An effort is being made at present to find necessary conditions for the existence of enclosures in systems with three or more dimensions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 08327-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mathematical Methods for Analysis of Large Scale Metabolic Networks

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. J. M. Park Expert LTB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Methods for analysis of metabolic networks based upon stoichiometry and hierarchical structure have been developed. Computer programs have been written which generate reduced equivalent networks for metabolic systems with steady state pools and rapid equilibrium reactions. New methods and computer programs for efficient solution of equilibrium networks have been produced.

Project Description

Objectives: To develop mathematical methods which will aid in the analysis, simulation and conceptual understanding of large scale metabolic systems. The development of metabolic network theory.

Major Findings: A new network theory approach has been developed based upon the stoichiometric concept. This allows the development of hierarchical structures of metabolic systems and provides numerical procedures which are far more efficient than standard solution methods. These concepts have been used to produce an equilibrium subnetwork solver which can rapidly solve for any range of reactants and equilibrium constants which can occur in metabolic systems.

Significance to Biomedical Research and the Program of the Institute: Will aid in the achievement of Objective 4, Approach 4 of the National Cancer Plan. Has an across-the-board application to all problems which depend upon facility in the mathematical analysis of biochemical systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08328-01 LTB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Systematic Deviations in Regressions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Van Eerdewegh	Visiting Fellow	LTB	NCI
OTHER:	M. Berman, Ph.D.	Acting Chief	LTB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Theoretical Biology

SECTION

Mathematical Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Linear regression analysis on weighted residuals is proposed to detect systematic deviations. The choice of the regressors is arbitrary and allows us to test for any particular trend: linear, polynomial, etc.

Project Description:

Objectives: We want to develop and implement in the SAAM Program criteria for detecting systematic deviations occuring in the process of fitting data by linear or non-linear models.

Major Findings: Linear regression analysis on weighted residuals is proposed to detect systematic deviations. The choice of the regressors is arbitrary and allows us to test for any particular trend: linear, polynomial, etc.

Significance to Biomedical Research and the Program of the Institute: The methodology of modeling is most essential for studying the behavior of complex systems for both normal and abnormal states.

IMMUNOLOGY BRANCH

Introduction

I. Structure and Function of Immune Cell and Neoplastic Cell Membranes and Cell Products

- A. Membrane Receptors
- B. Mechanisms of Cell Killing
- C. Normal Cell Surface Antigens
- D. Modified Cell Surfaces

II. Cellular Immunity

- A. Separation and Characterization of Lymphoid and Macrophage Cell Populations
- B. Characterization of Lymphoid Populations in AKR Mice
- C. Modulation of Lymphocyte Circulation
- D. Regulation of the Immune Response

III. Genetics of the Immune Response

- A. Induction of In Vitro Cell Medicated Immunity Against Hemopoietic Histocompatibility Antigens
- B. Genetic Control of Responses to Altered Cell Surface Antigens
- C. Relationship Between Ir Genes and Immunoglobulin Variable Region Genes

IV. Transplantation Immunobiology

- A. Major Histocompatibility Locus Antigens of Mice
- B. Receptors for Transplantation Antigens
- C. Transplantation Antigens of Swine
- D. Preclinical Bone Marrow Transplantation
- E. HLA and Disease Associations

V. Structure and Immunochemistry of Immunoglobulins

VI. Clinical Immunotherapy

SUMMARY

Annual Report of the Immunology Branch, National Cancer Institute
July 1, 1975 through June 30, 1976

INTRODUCTION

The Immunology Branch carries out basic immunobiology laboratory investigations with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation. The fundamental information obtained is then applied to investigations of the interactions between the immune system and neoplastic cells, as well as to the possible diagnosis and therapy of malignant disease in animal models and in man.

I. STRUCTURE AND FUNCTION OF IMMUNE CELL AND NEOPLASTIC CELL MEMBRANES AND CELL PRODUCTS

A. Membrane Receptors. The function of cells, including cells of the immune system, is partially initiated and modulated by interactions between cell surface receptors and a variety of ligands. Recent studies have shown that Fc receptors, which can bind antigen-antibody complexes or aggregated IgG, are present on murine B cells, a subpopulation of murine T cells and macrophages. The Fc receptors of B cells and T cells (but not macrophages or "third population" lymphocytes) are associated with cell surface Ia antigens, but are not identical with them, since capping most Fc receptors of B cells does not alter the distribution of Ia cell surface antigens (5035). Antigens determined by a non-H-2 locus are also associated with the Fc receptor.

Fc receptors may play a key role in immunoregulation, since it has been shown that mitogenic stimulation of cells bearing Fc receptors can be inhibited by immobilized antigen-antibody complexes (5060). Production of polyclonal antibody by lipopolysaccharide stimulation of B cells also is suppressed by immobilized antigen-antibody complexes. The mechanism of these inhibitions is under investigation.

Attempts are being made to isolate and characterize the Fc receptor (5048). Experiments have led to inconsistent results and additional experiments will be carried out in the coming year.

B. Mechanisms of Cell Killing. Studies are being performed in an attempt to understand the mechanisms by which lymphocytes kill target cells (5018). Lipid bilayer membranes have been used as a model for the target cell membrane. When these model membranes contain DNP groups, and antibodies for DNP are allowed to interact with the membranes, subsequent addition of non-immune human lymphocytes causes a conductance increase of several orders of magnitude across the antibody coated lipid bilayers. These results suggest that an increase in ionic permeability may be the basic mechanism responsible for target cell death, at least in the antibody dependent cell mediated killing

system. In order to define the nature of the lesions inflicted by attacking lymphocytes, ionic selectivity was studied. Results indicate that lymphocytes alter lipid bilayer conductance in a matter different from previously studied ionophores. It appears that killer lymphocytes create lesions large enough to allow sizable molecules to pass freely through the membrane and methods other than electrical conductance will be required to study the size of these channels.

Previous studies in the antibody dependent cell mediated killing system have shown that attacking cells undergo a marked shape change when they interact with target cells (5018). Lymphocytes that interact with antibody coated plastic surfaces undergo identical rapid and dramatic morphologic changes. These changes, and the capacity of these cells to kill in the antibody mediated system, can be inhibited by drugs affecting energy metabolism, microtubules, microfilaments and intracellular cAMP levels. This implies that shape changes, especially flattening, may be an important aspect of the killing process. An electron microscopic study revealed that cells bearing Fc receptors contain bundles of macrotubules in their cytoplasm. These bundles are composed of tubulin and may play a role in uropod formation.

C. Normal Cell Surface Antigens. Human HLA antigens have been further characterized (5006). Amino acid sequence studies of the 20 N-terminal residues of two HLA antigens controlled by different genes have revealed good homology, consistent with the idea that the antigens represent the product of a gene duplication.

A human B lymphocyte antigen system has been identified and shown to have properties similar to those of murine Ia antigens, including linkage to the major histocompatibility complex. In a separate approach to the same problem, anti-HLA antisera were shown to contain antibodies that bind to human B cells and inhibit binding of antigen-antibody complexes to B lymphocyte Fc receptors (5034). Thus, two different approaches to defining Ia like antigens on human B cells have been successful and should provide reagents that will make it possible to define the functional significance of these antigens.

D. Modified Cell Surfaces. Continued studies have failed to reveal a biologic function for the antigen revealed when cells are treated with neuraminidase (5017). Similarly, no function has been found for the naturally occurring antibody that reacts with neuraminidase revealed antigens. The titer of this antibody appears to rise after influenza infections.

Tumors of C57Bl mice that do not stimulate cytotoxic antitumor activity by C57Bl spleen cells in vitro have been treated with sulfanilic acid (5003). Modified tumor cells have the capacity to stimulate specific antitumor activity against unmodified tumor cells. This may provide an approach to effectively immunizing patients against their own tumors.

II. CELLULAR IMMUNITY

A. Separation and Characterization of Lymphoid and Macrophage Cell Populations. Lymphoid cell populations have been fractionated on nylon

columns. Nonadherent cells (T enriched) and adherent (B enriched) synergize in the in vitro generation of cell mediated cytotoxicity to syngeneic TNP-modified cells (5039).

Adherent murine peritoneal cells were found to be quite heterogeneous in their effects on tumors (5054). The effect of adherent cells on tumors in vitro ranged from inhibition of tumor proliferation to profound stimulation of proliferation. Adherent cells from BCG treated mice were less stimulatory and also showed defective phagocytosis. This appeared to be due to increased numbers of adherent, but nonphagocytic, cells. Techniques were devised for the isolation of these cells. They were shown to have potent anti-tumor activity and characteristics of both macrophages and B cells. They probably represent a previously undescribed population of lymphocytes.

Suppressor cells may play a role in the induction or evolution of malignancies. Peripheral blood cells from patients with metastatic tumors do not generate cytotoxic cells when stimulated in vitro with mouse cells. When these human lymphocytes are prefractionated on nylon columns, normal responsiveness is restored (5003). Thus it is possible to remove a population of cells (presumably suppressor cells) that cause this lack of responsiveness.

Suppressor cells have also been identified in the spleens of tumor bearing AKR mice during the progressor phase of growth of a transplantable syngeneic tumor that spontaneously regresses (5051). The adherent spleen cells suppress mitogenic responsiveness, mixed lymphocyte reactivity and ability to generate cytotoxic effector cells when added back to normal spleen cells. Analysis of this system should give a better understanding of immune mechanisms controlling tumor growth.

Suppressor cells have been generated by in vitro incubation of normal mouse spleen cells alone without any intentional antigenic stimulus (5055). These suppressors are nylon adherent splenic T cells and they nonspecifically suppress in vitro induction of cell mediated responses to alloantigens, "modified cell" determinants and syngeneic tumors. No evidence of soluble suppressive factors have been found.

The fluorescent activated cell sorter (FACS) has been used to identify a population of murine B lymphocytes that play a critical role in the generation of humoral immune responses (5062). This population may be analogous to Ig bearing lymphocytes of man.

B. Characterization of Lymphoid Populations in AKR Mice. An attempt is being made to define the defect in the lymphoid system of AKR mice which predisposes to the high spontaneous incidence of thymic lymphoma and lymphocytic leukemia (5041). It has previously been shown that the spleens and lymph nodes of leukemic animals contain an increased proportion of theta positive lymphocytes. Normal or preleukemic mice do not show such a disproportion of theta positive cells. Analyses of mitogen responsiveness of lymphoid organs in leukemia AKR mice show that spleen cells have decreased PHA and Con A responsiveness, but that there is a more severe depression in Con A responsiveness causing an increase in the ratio of PHA to Con A mitogenesis. By

contrast, the thymoma cells show a marked absolute increase in PHA responsiveness. It appears that the changes in mitogen responsiveness of lymphocytes from leukemic AKR mice may reflect differential susceptibility of subpopulations of cells to infection by murine leukemia viruses. Tumors arising in aged AKR mice who are thymectomized at one month of age show cell surface characteristics of both T and B cells (5043). When passaged into young AKR mice, the cells express only T cell characteristics, but then differentiate and express both T and B cell markers. Factors controlling expression of these markers are being studied.

AKR mice are also being studied in terms of susceptibility to spontaneous and transplanted AKR lymphomas (5053). For reasons that are not clear, lymphoma cells that arise in AKR/J animals are rejected by AKR/Cu mice. These two strains of AKR differ in their ninth chromosome and the only known antigenic consequence is a difference in Thy antigens. The nature of the strong resistance to this tumor is being studied.

C. Modulation of Lymphocyte Circulation. The pattern of lymphocyte circulation or "traffic" through the lymphoid organs is altered by BCG (5042). Increased trapping of labelled syngeneic lymphocytes by spleen and draining lymph nodes is caused by intravenous or subcutaneous administration of BCG. BCG also alters the pattern of trapping of lymphocytes exposed to sheep red cells *in vitro*. The known adjuvant effects of BCG may in part be due to alterations of lymphocyte trapping in lymphoid tissues.

D. Regulation of the Immune Response. Antigen specific T cell factors for TGAL have been produced as part of a study of the role of the factors in T-T and T-B cell cooperation (5059).

Precise genetic differences required to generate proliferation or specific cell mediated immunity have been studied with congenic mice differing only in limited portions of the major histocompatibility complex (MHC). Proliferation and cell mediated cytotoxicity can be produced with differences limited to the D end of the MHC and in fact can be produced by a point mutation in the D region (5057). This confirms the prior observation that very limited alterations in genetic regions other than the I region can lead to significant sensitization.

III. GENETICS OF THE IMMUNE RESPONSE

The demonstration of genetic control of immune responses has opened an important pathway in gaining an understanding of the biology of the immune system. A variety of studies are being carried out in an attempt to determine the mechanisms of the genetic control as well as to determine the biologic significance of this type of control.

A. Induction of In Vitro Cell Mediated Immunity Against Hemopoietic Histocompatibility Antigens. Hemopoietic histocompatibility antigens are phenotypically expressed only on blood forming and leukemic cells and they are capable of stimulating host versus graft reactions which are radioresistant *in vivo*. An *in vitro* system in which F1 responding lymphocytes generate

cytotoxic effector cells against spleen cells of H-2^b parents but not against H-2^a parents has been developed (5049). Antigenic differences detected in this system are controlled by the H-2D-Hh-1 region of the MHC. Both in vitro and in vivo studies indicate that macrophages are the effector cells in this system and that different mechanisms are involved in the generation of immune responses to Hh and alloantigens. This system provides an in vitro model for elucidating the recognition and effector phases of hybrid resistance to parental hemopoietic grafts and may be useful in developing matching procedures for clinical bone marrow transplantation

B. Genetic Control of Responses to Altered Cell Surface Antigens. Cell mediated immunity can be induced in vitro against hapten modified target cells. This system demonstrates two aspects of genetic control. It has been shown that the antigen developed on the cell surface consists of a hapten modified protein which is itself an H-2 antigen under the genetic control of either the D or K end of the H-2 complex (5045). The immune response is not directed against the hapten itself but rather against hapten-modified cell surface proteins. Data suggest that the antigen recognized by the effector cells includes an altered H-2 cell surface protein and that there is a requirement for homology between stimulating and target cells at either or both ends of the major histocompatibility complex if lysis is to occur. The nature of the interactions generated by H-2 homology are not yet known.

The capacity to develop an immune response to the hapten modified cell surface protein is also under genetic control. Genes controlling this responsiveness are H-2 linked and mapped to the left of the Ia genetic region (5038).

C. Relationship Between Ir Genes and Immunoglobulin Variable Region Genes. Studies are being carried out to determine whether Ir genes control the immunoglobulin variable regions that are produced in "genetically controlled" antibody responses (5036). It has been shown that idiotype expression on mouse anti-nuclease antibodies is linked to heavy chain allotype markers and not to the immune response gene locus. Further studies of this system will explore the relationship of idiotype to T cell recognition.

IV. TRANSPLANTATION IMMUNOBIOLOGY

A. Major Histocompatibility Locus Antigens of Mice. Antigens (Ia antigens) determined by genes in the Ir region of the MHC have been shown to be a major source of stimulation of the mixed lymphocyte reaction (5021). Antisera directed against Ia antigens block the binding of antigen-antibody complexes to the Fc receptor of B cells and a portion of T cells, suggesting that Ia antigens and Fc receptors are physically close to one another on the surface of cells (5021, 5035). Anti-idiotypic antisera are being prepared and will be used to further analyze and modulate in vivo graft rejection.

The structure of Ia antigens is being analyzed (5047). An improved method for indirect immunoprecipitation has been developed. Utilizing this improvement, analyses of Ia antigens indicate that antigenic specificities determined by alleles of the I-A region are located on separate molecules.

Similarly, specificities controlled by genes located in different portions of the I region (e.g., those in I-A compared to I-C) are always found on separate molecules. Assuming that this will be true in all instances, chemical determinations can be used to provisionally "map" the probable location of genes determining new antigens as they are found.

B. Receptors for Transplantation Antigens. Cytotoxic cells, generated by in vitro allogeneic sensitization, can be adsorbed by monolayers of the immunizing H-2 type (5056). Unsensitized cells could not be depleted of cytotoxic cell precursors by this type of adsorption and in fact, developed an augmented response after adsorption. This suggests that receptors on precursor cells are in a state not capable of binding efficiently to cells of the appropriate specificity. Additional attempts will be made to find ways to specifically deplete these precursor cells.

C. Transplantation Antigens of Swine. Three herds of miniature swine have been bred to the point where they are homozygous for the histocompatibility antigens at the MHC (5023). In vitro assays and skin graft results all indicate homogyzosity. Antisera detecting antigens of the MHC have been prepared and are being used to serologically and chemically analyze the products of the MHC.

D. Preclinical Bone Marrow Transplantation. Rhesus monkey alloantisera have been produced and evaluated (5011). Six new MHC antigens have been defined in the course of these investigations. Two animals who appear to be homogyzous at the genetic locus determining MLC stimulation have been identified and their cells are being used to type families and other populations for MLC antigens.

E. HLA and Disease Associations. HLA antigens have been associated with gluten-sensitive enteropathy and dermatitis herpetiformis (5017). Prospective studies of acute leukemia and lung cancer patients are in progress.

V. STRUCTURE AND IMMUNOCHEMISTRY OF IMMUNOGLOBULINS

Studies of the structure and function of the constant portions of immunoglobulins are in progress (5050). The nature of the interaction between the constant portion of rabbit antibodies and complement components is being analyzed. In addition, divalent affinity labelled antigens have been used to create antigen-antibody complexes of small defined size and the ability of these complexes to bind to cell surface Fc receptors is under investigation.

VI. CLINICAL IMMUNOTHERAPY

A protocol comparing chemotherapy and two different forms of immunotherapy in patients with stage I and stage II malignant melanoma has been initiated (5033). Almost 50 patients have entered this randomized trial but it is too early to assess results. Serum and lymphocytes are being obtained from these patients before they begin their therapy and then periodically during the course of therapy. In vitro assays are being performed with serum and cells to evaluate alterations in specific immune responsiveness (5016).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5003(c)-10 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Immunity I

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Wunderlich, M.D.	Senior Investigator	IB NCI
OTHER:	A. E. Eggers, M.D.	Clinical Associate	IB NCI
	H. S. Koren, Ph.D.	Visiting Fellow	IB NCI
	C. A. Janeway, M.D.	Staff Associate	LI NIAID
	J. K. Inman, Ph.D.	Senior Investigator	LI NIAID
	D. M. Segal, M.D.	Investigator	IB NCI
	G. M. Shearer, Ph.D.	Senior Investigator	IB NCI

COOPERATING UNITS (if any)

Barry S. Handwerger, M.D., University of Minnesota, School of Medicine, Minneapolis, MN.

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

SUMMARY OF WORK (200 words or less - underline keywords)

1) The deficient cellular immune responsiveness of lymphocytes from patients with widespread malignant disease can be corrected by fractionation of these cells before in vitro stimulation.

2) Tumor immunogenicity has been increased in a mouse model system by chemical modification of the tumor cells.

Project Description:

Objectives: This project has been directed at understanding and manipulating mechanisms of cell-mediated immune cytotoxicity in both humans and mice. Long-range goals are: (1) to identify and characterize factors which influence induction, maturation, and expression of cytotoxic cellular immune responses; (2) to define changes in cytotoxic cellular immunity which occur in tumor bearing hosts; and (3) to find means of manipulating the cytotoxic cellular immune response for therapeutic purposes. Immediate objectives of the past year have been to concentrate on goals #2 and 3.

Methods Employed: Cell-mediated cytotoxicity is measured in vitro by release of ^{51}Cr from labelled target cells incubated with lymphoid cells for 2-4 hours. Immune cells are collected from spleen, marrow, lymph node, thymus and peripheral blood of sensitized hosts. Several techniques have been used for fractionating cells. Spleen and peripheral blood cells have been fractionated with Ficol-hypaque gradient centrifugation and on nylon columns; effluent cells from the nylon columns are highly enriched for T lymphocytes. In addition, immune cells have been produced by in vitro sensitization of spleen and peripheral blood cells using previously established Mishell and Dutton tissue culture techniques. Target cells are provided by freshly explanted cells, tissue culture lines, and established tumors passed in vivo or in vitro. Target cells have also been chemically modified in order to change the immunogenicity of cell surface antigens.

Major Findings: 1) While working at the Clinical Research Center in Harrow, England, one of us (JW) has shown that PBL from each of 8 humans with widely metastatic tumor failed to generate cytotoxic cells in vitro when stimulated with mouse cells. Mouse cells were chosen for this test because of previous knowledge that PBL from normal humans do respond well in vitro by generating cytotoxic cellular immunity. The major finding has been that nylon column fractionation of PBL from tumor patients before sensitization renders the in vitro response normal.

2) Two independent tumors from C57BL mice have been altered by treatment with sulfanilic acid. Unmodified tumors have failed to stimulate in vitro production of cytotoxic antitumor activity by C57BL spleen cells. Modified tumors, however, have stimulated generation of T cells which are specifically cytotoxic for the unmodified tumor.

Significance to Biomedical Research and the Program of the Institute: 1) Previous investigations have clearly shown that PBL from patients with widespread tumor metastasis are deficient in a variety of immunologic functions, such as mitogen responsiveness and mixed lymphocyte reactions. At least one function, generation of cytotoxic cells, can now be improved to normal levels by nylon-column fractionation of responding lymphocytes before in vitro stimulation with foreign cells.

2) Chemical modification of tumor cells by sulfanilic acid is a promising step toward stimulating cell-mediated immunity against syngeneic and autologous tumors. Combining this procedure with nylon-column fractionation of responding cells appears to be our optimal approach toward generating antitumor immunity in vitro, which will be useful not only for understanding the nature of cellular immunity to tumor cells, but also for passive immunotherapy.

Proposed Course of Project: Primary effort will be directed at increasing the in vitro cytotoxic T cell response against syngeneic and autologous tumors. This work will involve both mouse and human tissues. In addition we will continue our study of human cells which mediate antibody-dependent cytotoxicity.

Publications:

Eggers, A.A.: Tumor specific immunogenicity induced by chemical modification. J. NCI, in press.

Janeway, D.A., Koren, H.S. and Paul, W.D.: The role of thymus derived lymphocytes in an antibody mediated, hapten specific helper effect. Eur. J. Immunol. 5:17-23, 1975.

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Koren, H.S., Wunderlich, J.R. and Inman, J.K.: T cell memory for the cytotoxic response to hapten-modified target cells. J. Immunol. 116:403-408, 1976.

Rehn, T.G., Shearer, G.M., Koren, H.S. and Inman, J.K.: Cell mediated lympholysis of N-(3-Nitro-4-Hydroxy-5-Iodophenylacetyl)-β-Alanyl-Glycyl-glycyl-modified autologous lymphocytes: Effector cell specificity to modified cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. J. Exp. Med. 143:127-142, 1976.

Eggers, A.A.: Immune response to an allogeneic progressively growing solid tumor. Cancer Res., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER NCI Z01 CBD 5006 (c)-10 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Lymphocyte Cell Surface Antigens, Normal and Neoplastic

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Mann, M.D.	Senior Investigator	IB NCI
OTHER:	J. L. Miller, Ph.D.	Staff Fellow	IB NCI
	P. J. Romano, Ph.D.	Postdoctoral Research Fellow	IB NCI
	B. G. Leventhal, M.D.	Head, Chemoimmunotherapy Section	PO NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	

SUMMARY OF WORK (200 words or less - underline keywords)

The biologic and chemical characteristics of lymphoid cell surface antigens are under investigation. A human B lymphocyte antigen system has been identified and the genetic control shown to be linked to genes controlling HL-A antigens. Mixed lymphocyte reactions can be inhibited by the antisera directed against B lymphocyte specific antigens.

Results to date indicate that the lymphocyte Fc receptor is not structurally related to the B cell antigens. In addition specific B lymphocyte antigens not found in the normal population have been identified in two disease states, glutin sensitive enteropathy and dermatitis herpetaformis.

Complete homology of the first 20 N terminal amino acids have been found in 2 HL-A antigens controlled by different genes. Preliminary results of studies designed to determine the molecular and chemical nature of the B lymphocyte antigens has shown these antigens to be different in size from the major histocompatibility antigens HL-A. Patients with acute myelocytic and acute lymphocytic leukemia were immunized with a long term tissue culture cell line, RAJI, with or without BCG adjuvant therapy. All parameters of immune response to leukemia associated antigens increase in patients receiving the RAJI-BCG immunization.

Project Description:

Objectives: The objectives of these studies are as follows: a) to purify human cell surface antigens; b) to chemically, genetically and immunologically characterize these antigens; c) to use purified cell membrane substances for heterologous immunization; d) to relate the presence of cell surface antigens to disease states and investigate their functional and biological significance; e) to assess the biologic relationship of different cell surface structures in relationship to the immune function of lymphocytes. The objectives of the studies of neoplastic lymphocyte cell surface antigens are as follows: a) identification of the antigen or antigens associated with acute leukemia; b) to isolate and chemically characterize these antigens; c) to use antigens crossreactive with acute leukemia associated antigens to immunize patients with acute leukemia; d) to assess the immunologic response of these patients to such immunizations.

Methods Employed: ^{51}chromium release and dye exclusion cytotoxicity tests were used to evaluate cell surface antigens both on normal lymphocytes, subpopulations of lymphocytes and neoplastic lymphocytes. The cells used in these tests were obtained from normal donors, donors with various disease states, and long term human lymphoid tissue culture cell lines. A new test was developed for the detection of B lymphocyte alloantigens. In brief this assay employs the ability of Fc receptor bearing lymphocytes to adhere to antigen antibody complexes. Microtiter plates were coated with the antigen TNP and antibody to this antigen. The plates were washed and whole peripheral blood lymphocytes added. After a period of incubation to allow the Fc positive cells to adhere, the nonadherent cells were removed. These nonadherent cells were replaced for testing with the antisera. Both the nonadherent (T) and the adherent (B) cells were tested with the antisera. After incubation and washing, complement was added and the cytotoxicity measured by dye exclusion. Antisera used in these tests were those obtained from multiparous or heterologous sera raised by purposeful immunization. Antigens in subcellular form were detected by inhibition of cytotoxicity using either the dye exclusion test or the ^{51}chromium release employing the antisera and the test systems described above.

Mixed lymphocyte reactions were performed by the standard procedure using mitomycin as an inhibitor of DNA synthesis in the stimulating cells. Peripheral lymphocyte and/or long term tissue cell lines were employed in these studies. It was established that B cells caused lymphocyte stimulation. B cell specific antisera were then incubated in the presence of the stimulating cell to assess the ability of these antisera to block the mixed lymphocyte reaction.

Lymphocyte dependent antibody studies were performed using peripheral blood lymphocytes attacking cells, these same cells as target cells or long term tissue culture lymphoid cells as targets. The target cells were labelled with ^{51}chromium and incubated with appropriate antisera. Attacking

cells were added and after four hours incubation, the release of the ^{51}chromium was assessed. Studies performed to assess the relationship of the Fc receptor to specific B cell alloantigens were accomplished by precoating the attacking cells with heterologous sera prepared specifically against B cell alloantigens and the lymphocyte dependent antibody test performed in the manner described above.

Patients with acute lymphocytic leukemia were immunized with 10^8 RAJI cells twice weekly after the patients had been induced into remission with chemotherapy. In one half of the patients the RAJI cells were injected together with BCG and the other half of the patients RAJI cells were injected alone. Sera from these patients were tested for their ability to react in cytotoxicity assays with the immunizing cell, RAJI, as well as against autologous leukemic cells. Sera were also screened for anti HL-A antibodies in the dye exclusion assay. Peripheral blood lymphocytes from these patients were tested for their ability to cause direct cytotoxicity to the immunizing cell as well as to the acute leukemia cells. The peripheral blood lymphocytes from the patients post immunization were assessed for their ability to cause cytolysis of leukemia cells, normal cells and the immunizing RAJI cells.

Lymphocyte membrane alloantigens were prepared by the following method. Lymphoid tissue culture cell lines were lysed with hyotonic solutions and membrane fragments isolated by gradient centrifugation. Cell membranes were then treated with non-ionic detergents or the enzyme papain. After a suitable incubation period insoluble material was removed by centrifugation and the solubilized cell membrane substances pooled and concentrated. Purification of the active constituents of these preparations was done on gel filtration and ion exchange chromatography, isoelectrofocusing, and acrylamide gel electrophoresis. Purity of the specific antigenic component was determined by labelling lymphocyte membranes with ^{5}H amino acids. The purified soluble antigens were complexed to antisera detecting a specific antigen and its purity assessed by the changes in size of the labelled material. Amino acid sequence analysis was performed on the Beckman 890B automatic sequencer. Carbohydrate analysis was performed using gas chromatography.

The quantities of lymphoid cells needed to pursue the immunotherapy protocol and to perform chemical structure studies on the human histocompatibility antigens were provided by the NCI contract NO1-CB-12078.

Major Findings: Milligram quantities of purified HL-A antigen cell membrane products have been prepared. Products of the HL-A or HL-B genetic locus were isolated and subjected to an analysis of their amino acid composition. In addition initial sequence studies have begun on these isolated antigens. The distribution of different amino acids in the products from separate genetic loci were nearly identical. The sequence of first 25 N-terminal amino acids of the HL-A2 antigen from two different sources was identical. The amino acid sequence of the product of the HL-B locus, HL-A7,

showed similarities to the HL-A2 antigen (HL-A genetic locus) through the first nine N-terminal residues. Small variations in amino acid sequence were found in the remainder of the amino acids determined through the 20th amino acid. The carbohydrate composition of carbohydrate moiety of this glycoprotein was identical in all purified preparations from the products of the HL-A and HL-AB genetic locus.

Serologic identification of B cell antigens in a number of large families were determined. The results demonstrated that B cell antigens followed the inheritance pattern of the major HL-A antigens and thus the genetic control appears to be linked to the genetic loci controlling the expression of HL-A alloantigens. The sera used in these studies did not react with human peripheral blood T cells. B cell antigens were determined in a family wherein a genetic crossover between the HL-A and HL-AB locus had occurred. The study in this family demonstrated that at least two genes were controlling the expression of the B cell specific antigens.

Peripheral blood lymphocytes from patients with glutin sensitive enteropathy and dermatitis herpetiformis were tested for B cell specific antigens. Serologic reagents used in these studies were those that had previously been used to identify the B cell antigens in family studies. In addition maternal sera were obtained from the appropriate relatives of the patients with these diseases. Two sera identified B cell specific antigens that were associated with the above disease states. These antigens were not found on the peripheral blood B cells from a randomly selected normal population. Six families were studied in which one member of the family had one of the diseases stated above. The B cell antigen specific for this disease was consistently found in the parents of children with the disease. However the inheritance pattern of this antigen suggested that the genetic control of this B cell antigen is not linked to the expression of HL-A and alloantigens. The wheat germ protein glutin which precipitates the gastrointestinal lesions in these diseases was tested in inhibition assays using cells from the patients and the sera detecting the B cell specific antigens of these diseases. This protein inhibited the cytolysis of the B cells with these antisera. Studies were performed which suggests that this B cell antigen is the receptor for the wheat germ protein glutin.

Heterologous antisera raised against different molecular weight components of B and T cells were tested for their ability to inhibit or interfere with the biologic function of peripheral blood lymphocytes. Antisera directed against a molecular weight component of 29,000 and 23,000 (papain prepared) was found to react with Ig positive B cells as well as Ig negative Fc positive null cells. These sera inhibited the function of the null cells to act as destructive attacker cells in lymphocyte dependent antibody reactions. It was determined that this inhibition was due to killing of the attacker cells. This finding was confirmed when no inhibition was observed when Fab_2 fragments were made of this antisera. In addition the heterologous antisera were found to inhibit the mixed lymphocyte reactions.

The human lympoid cells in long term tissue culture with characteristics of either T or B cells were studied for their ability to stimulate in mixed lymphocyte reactions. The lymphoid cells in culture with B cell characteristics were found to stimulate in the mixed lymphocyte reaction while the T cell counterparts were found not to stimulate. The alloantisera directed against the human B cell alloantigens were found in some instances to inhibit the stimulatory activity of the B lymphoid cell lines as well as the allogeneic stimulation of peripheral blood lymphocytes. The studies suggest that these sera in some instances are directed at the cell membrane product of the MLRS locus, therefore, inhibiting the mixed lymphocyte reaction. Studies with the heterologous sera appeared to demonstrate that the Fc receptor is not genetically determined by those genes controlling the expression of the human B cell alloantigens. Spatial arrangements on the lymphoid cell surface may account for some apparent inhibition of functional activity of these lymphocytes.

Immunization of patients with either acute lymphocytic or acute myelocytic leukemia were carried out with the lymphoid cell line RAJI with or without combined adjuvant therapy of BCG. Patients receiving the combination of the RAJI cells and BCG were found to respond in a more rapid fashion by several immunologic parameters. Earlier development of antibody directed against immunizing cell source was found when BCG was used. In addition an earlier rise in mixed lymphocyte response reactions were found in these patients compared to patients immunized with the RAJI cells alone. Skin tests reactivity developed more rapidly as did direct cellular cytotoxicity. Patients immunized with BCG and RAJI appeared to have longer remission duration, however the small numbers of individuals in each arm of the protocol does not allow for statistical evaluation of apparent effect.

Significance to Biomedical Research and the Program of the Institute: Structural analysis of the HL-A alloantigens approaches several fundamental biologic questions. The protein structure of the cell membrane components on lymphocytes is unknown. The HL-A antigenic markers provide the necessary resource for the isolation and purification of a specific cell membrane structure. Structural analysis of these cell membrane components will provide information as to the nature of changes that occur in these cell membrane structures that determine their individual antigenicity. Cell membrane structural changes as well as antigenic changes are known to occur in neoplasia. Structural studies on normally determined genetic products thus provide a prototype for studies of antigenic changes that occur with neoplastic transformation.

The human B lymphocyte antigens appear to be analogous in many respects to the B-cell alloantigenic system in mice. Murine B cell alloantigens appear in some way to be connected with the ability of the animal response to a particular antigen. Identification of B cell alloantigens in man allows similar investigation of this immunologic phenomenon. An increasing number of disease states have been found to be associated with the expression of

certain HL-A alloantigens. Certain diseases have a very high association with particular HL-A alloantigens. However, not all individuals with a particular HL-A alloantigen have any particular disease. This suggests that there may be genes, closely linked to the major histocompatibility antigens, which participate in some fashion in either the pathogenesis or the expression of the disease. B cell alloantigen typing provides a mechanism to identify these antigens. The studies in gluten sensitive enteropathy and dermatitis herpitiformis provide a prototype for the investigation of disease states associated with the expression of HL-A alloantigens.

The cell membrane structures of human lymphocytes may serve as receptors for the initiation of the immune response as well as participate in the efferent limb of immune reactivity. Identification of these receptors, as well as the determination of their biologic activity, are important fundamental biologic questions.

Proposed Course of Project: We have demonstrated that acute leukemia cells possess specific antigenic determinants. This gives a rational basis for immunotherapeutic trials. The studies thus far have demonstrated an immune response in patients with acute leukemia immunized with the RAJI cell line. This is demonstrated by the development of antibody reacting specifically with leukemia cells. Immunotherapeutic trials will be continued with assessment of immunologic response and remission duration.

Amino acid sequence studies of these isolated purified HL-A and HL-B products will continue. In addition, these studies will be extended to the alloantigens specific for B lymphocytes. Genetic and molecular correlations will be made.

B lymphocyte alloantigens will be determined in a variety of disease states particularly those which have previously shown to be associated with HL-A alloantigens.

Studies are underway to determine the molecular and spatial relationship of different lymphocyte cell surface components and to relate those to the afferent and efferent limb of the immune response.

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Yust, I., Smith, R.W., Wunderlich, J.R. and Mann, D.L.: Temporary inhibition of antibody-dependent, cell-mediated cytotoxicity by pretreatment of human attacking cells with ammonium chloride. J. Immunol., in press.

Strominger, J.L., Chess, L., Humphresy, R.E., Mann, D.L., Parham, P., Robb, R., Schlossman, S., Springer, T. and Terhorst, C.: Isolation and structure of products of the human histocompatibility gene complex. In Katz, D. (Ed.): Proceedings of "The Role of Products of the Histocompatibility Gene Complex in Immune Responses". In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5011 (c)-06 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Rhesus Monkey Histocompatibility Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. R. Neefe, Jr., M.D. Investigator IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

150 rhesus monkey alloantisera raised by planned immunization or received from other laboratories were evaluated for lymphocytotoxic antibodies by (1) titration against peripheral blood lymphocytes of selected monkeys; (2) typing of 112 random unrelated monkeys and of members of 30 monkey families by standard microcytotoxicity methods. This resulted in an increase of RhL-A serologically defined antigens which we recognize from 11 to 18 including 7 in the first series and 11 in the second. Quality of sera defining antigens has been greatly improved in each case since (1) frequency of defining sera for each group is very close; (2) X^2 of correlation of sera in pairwise comparison of defining sera for each group is very high. One possible third series antigen is being evaluated.

Two monkeys have been identified which behave as LD typing cells or homozygotes at the major locus determining MLC stimulation (LD). These are being used to type families and random unrelated populations for the 2 LD antigens.

Breeding to produce RhL-A homozygotes and RhL-A identical siblings is producing about 10 live births per year.

Project Description:

Objectives: To establish and maintain a colony of pedigreed rhesus monkey families for study of the genetics of transplantation and as an eventual source of donor-recipient pairs for transplant experiments.

This work is being conducted at Litton-Bionetics under contract NO1-CB-64016. Dr. Bruce Maurer is the Principal Investigator. Dr. Neefe is the Project Officer.

Methods Employed: Sera are raised by immunization with skin graft and peripheral blood lymphocytes of selected monkeys previously typed for RhL-A major histocompatibility complex antigens and appropriately matched to related or unrelated donors. Sera are analyzed for serologically defined antigens by titration against known positive and negative monkeys, by microcytotoxicity typing of families and large random unrelated populations and by standard statistical analysis of patterns of reactivity observed. Selected sera are further analyzed for monospecificity by absorption and titration.

Monkeys are typed for MLC locus (LD) antigens by performing mixed lymphocyte cultures using as stimulators the two available monkeys homozygous at this locus. These monkeys are among the progeny of a single male and represent both of that male's LD antigens.

Breeding is designed to produce RhL-A homozygotes, RhL-A identical siblings, and to enlarge existing families of full siblings.

Major Findings: Analysis of about 150 alloantisera has resulted in improved definition or new identification of a total of 18 RhL-A alloantigens including 7 in a 1st series and 11 in a 2nd. (11 antigens were previously identified by sera available.) All except 2 antigens are defined by more than 1 serum; in most cases the sera defining a group have very similar frequencies and are very highly correlated ($p<.00001$) in X^2 2 x 2 analysis. An antigen possibly belonging to a third linked locus is being evaluated.

The MLC homozygotes are being evaluated to establish RhL-A linkage of the MLC locus, frequency of the alleles in the population, linkage disequilibrium with any other RhL-A markers, and significance of the MLC locus for skin transplantation.

Breeding has resulted in about 10 live births including the RhL-A homozygote and two new RhL-A identical sibling pairs.

Proposed Course of Project: Major emphasis will be upon identification of MLC loci and upon development of antisera for these determinants. Efforts further to improve definition of the RhL-A serologically defined antigens by

raising and analyzing new sera and to expand the quantity and immunogenetic usefulness of the family members will continue.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5016 (c)-06 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Immunity II

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Wunderlich, M.D.	Senior Investigator	IB NCI
OTHER:	W. D. Terry, M.D.	Associate Director for Immunology	IB NCI
	R. J. Hodes, M.D.	Head, Immunotherapy Section	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	.5	2

SUMMARY OF WORK (200 words or less - underline keywords)

Patients participating in an NCI immunotherapy protocol are being tested for cytotoxic humoral and cellular immunity against melanoma vaccine cells. Increases in humoral but not cellular immunity have been associated with immunotherapy.

Project Description:

Objectives: The objective of this project has been to carry out *in vitro* tests for humoral and cellular immunity against melanoma vaccine cells. Patients being tested are associated with an NCI immunotherapy protocol.

Methods Employed:

1) Direct, Cell-Mediated Cytotoxicity. Cytotoxicity is measured by the release of ^{51}Cr from labelled target cells incubated with immune cells for 4 hours. Target cells are prepared from vaccine melanoma cell lines maintained in tissue culture. Normal and immune lymphoid-attacking cells are prepared from human peripheral blood. Peripheral blood cells are purified by centrifugation with Ficoll-hypaque. Studies of multiply transfused humans have shown that cytotoxicity in this assay is specific for direct cell-mediated reactions and independent of conventional complement, humoral antibody, and nonspecific toxins.

2) Lymphocyte Dependent Antibody (LDA) Mediated Cytotoxicity. This cytotoxic pathway is also measured by a chromium release assay. After ^{51}Cr labelling, melanoma target cells are washed by centrifugation and then incubated for 1/2 hour with heat-inactivated serum or plasma containing LDA. Incubation mixtures include carregeenan, a C1 attachment inhibitor. Target cells are again washed and then incubated with nonsensitized attacking lymphoid cells for 4 hours.

3) Complement-Dependent, Antibody-Mediated Cytotoxicity. The complement assay is a two-stage, ^{51}Cr-microcytotoxicity test utilizing rabbit complement and vaccine melanoma cell lines.

Major Findings: In vitro cytotoxicity tests have been carried out in order to monitor the response of melanoma patients to the immunotherapy vaccine. Last year 202 patient samples were screened for direct cell-mediated cytotoxicity, 79 samples for lymphocyte-dependent antibody, and 165 samples for complement-dependent antibody against the vaccine cell lines. Immunotherapy has been associated with development of complement dependent antibody against vaccine cell lines. Tests for other types of immunity against vaccine cell lines have failed to demonstrate increased activity associated with immunotherapy.

Significance to Biomedical Research and the Program of the Institute: *In vitro* cytotoxicity tests are being used to help assess the effectiveness of immunotherapy for melanoma patients. Although these tests may detect patient responsiveness to allogeneic transplantation antigens, they nevertheless serve the useful purpose of detecting nonresponsive patients or an ineffective vaccine.

Proposed Course of Project: The project will consist of continued evaluation of patients on the melanoma immunotherapy protocol by assessing humoral and cellular cytotoxicity immune responses to the vaccine cell lines.

This project now concerns only in vitro testing of patients on the melanoma immunotherapy protocol. Previous aspects of this project, directed at investigation of cellular immunity in humans, have been shifted to Project #NCI ZO1 CBD 5003(c)-09 I titled Cellular Immunity I.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD-5017(c) 05-I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological Significance of HLA and Neuraminidase Revealed Cell Surface Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. N. Rogentine, Jr., M.D.	Senior Investigator	IB, NCI
OTHER:	S. H. Pincus, M.D.	Clinical Associate	IB, NCI
	W. Strober, M.D.	Head, Immunophysiology	MET, NCI
	Steven Katz, M.D.	Senior Investigator	D, NCI
	W. Bunney, M.D.	Chief	AP, NIMH
	E. Gershon, M.D.	Senior Investigator	AP, NIMH

COOPERATING UNITS (if any)

Duke University, Contract NO1-CB-53890
Childrens National Medical Center, Washington, D.C.
Mayo Clinic, Rochester, Minnesota

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

SUMMARY OF WORK (200 words or less - underline keywords)

We have been studying the biological significance of both the antigen on human and murine tissue revealed by neuraminidase treatment and its antibody. In humans this antibody titer can be boosted by influenza virus infection. This virus possesses neuraminidase. We are investigating possible lymphocyte mitogenic properties of the antibody, and we are searching for the natural occurrence of the antigen on tumor tissue.

HLA antigens have been associated with a variety of diseases. Our studies concentrate on malignant diseases and disorders of immunity. We are testing the hypothesis generated by our previous work that long term survival in leukemia and lung cancer is due to the possession of certain HLA antigens. Other diseases studied include melanoma, gluten-sensitive-enteropathy, dermatitis herpetiformis, acute schizophrenia and manic-depressed psychoses.

Project Description:

Objectives: Definition of the natural biological significance of certain human cell surface antigens is our objective. We have studied two groups of antigens. The first is an antigen or set of closely related antigens revealed after neuraminidase treatment of intact cells. The second group is the well known HLA antigen system. Nothing as yet is known of the biological significance of the first group of antigens. The latter group has importance for organ transplantation, but our emphasis is upon the relationship of HLA antigens to the natural function of the human organism.

We are approaching the significance of the neuraminidase revealed antigen(s) by studying the chemical nature of the antigen, its distribution on different cells and its naturally occurring autoantibody.

We are seeking the significance of HLA antigens by investigating their frequency disturbances in different diseases. If such disturbances are found, and many have been, we can design further studies on the role of the antigen in the pathophysiology of disease.

Methods Employed: Cell surface antigens are assayed by standard ^{51}Cr release or dye exclusion cytotoxicity tests. Cells, usually lymphocytes, are either untreated or incubated with Vibrio cholera neuraminidase under conditions which do not affect cell viability but which remove most of the cell surface sialic acid. Estimates of antigen content of different cells are achieved by quantitative inhibition of antisera. HLA typing is being conducted on contract by Drs. F. Ward and A. H. Johnson of Duke University.

Major Findings: In the past year we have been studying three aspects of the neuraminidase revealed antigen problem. The first study was an analysis of serum antibody levels in subjects who had had influenza in the epidemic of 1974/1975. Influenza virus has an endogenous neuraminidase; natural infection could allow this enzyme to modify self antigens so that they would be recognized by the host's immune system, thereby provoking an antibody response. We hypothesized that subjects with natural influenze infections would have a rise in titer of the antibody to neuraminidase treated lymphocytes. We found this: in 25 acute-convalescent serum pairs from documented cases of influenza (provided by Dr. Noble), 9 (36%) showed a significant, ie. greater than fourfold, rise in titer in the convalescent serum. This rise was not correlated with the rise of antibody to other influenza virus antigens, as measured by Dr. Noble. In 43 pairs of normal and disease control sera, 2 (5%) showed a fourfold or greater change in titer over comparable time periods. The difference in titer rise between the influenza and the control serum pairs is significant at $p<.01$. Thus it would appear that natural infection with a neuraminidase containing microorganism, influenza virus, can result in increased antibody production to this antigen.

The other two studies in this area are in progress. One involves assaying various untreated normal and tumor tissues in humans and in mice for the presence of this antigen. There is some evidence that tumors may not add

terminal sialic acids to growing cell surface oligosaccharide chains. These incomplete sugar chains would be similar to those we created on normal cells by neuraminidase digestion. We are, therefore, assaying various malignant human and murine cell types for the native presence of this antigen.

The natural antibody has specificity for β-D-galactosyl non-reducing-end-oligosaccharides. Some lymphocyte mitogenic plant lectins, notably peanut and soybean, have similar specificity. These lectins are mitogenic only for neuraminidase treated lymphocytes. The natural antibody may itself act as a lymphocyte mitogen or, alternatively, it may block the mitogenic activity of these lectins. Experiments to test these possibilities are in progress.

Studies of the association of HLA antigens and disease continued in several areas. The HLA assays are done entirely under contract with Duke University. Most of the studies are in progress at present.

On the basis of previous work in acute leukemia and lung cancer, we hypothesized that the possession of certain HLA antigens conferred survival advantage to patients with these antigens. Testing this hypothesis requires the prospective typing of a large number of cancer patients. We are currently doing this. Through a collaboration with the Cancer Chemotherapy Study Group (previously Acute Leukemia Group A), we are studying all their newly diagnosed acute leukemia cases. At over 300 patients already typed, this is the largest series ever studied. We plan to ask two questions: a) Are HLA antigen frequencies abnormal at diagnosis (implying a susceptibility/resistance factor to the development of the disease); and b) Does survival correlate with possession of certain HLA antigens.

A similar study of stage I lung cancer patients is being conducted with the NCI Lung Cancer Project at the Mayo Clinic.

We are continuing to type melanoma patients and to assay post immunization (with a killed allogeneic melanoma cell vaccine) sera for anti-HLA activity.

With Dr. W. Strober we have typed over 30 families of patients with sprue. Although HLA-B8 (old HL-A8) is highly associated with this disease, our family studies indicated that at least one other gene on a separate linkage group is needed to explain the occurrence of this disease. In this regard it is interesting that Dr. D. Mann (IB, NCI) and Dr. Strober have found a non-HLA linked B lymphocyte alloantigen in nearly all of the sprue patients.

With Dr. Katz, NCI, we assayed HLA antigens in dermatitis herpetiformis patients who had IgA skin deposits, perhaps the best diagnostic criterion in this disease. 85% (28 of 33) had HLA-B8. Previous studies from our laboratories had shown about a 60% incidence of HLA-B8 in this disease when the IgA skin deposit criterion was not applied. The normal HLA-B8 frequency is 20%.

We are continuing to assay HLA antigens in patients with well documented acute schizophrenia, since there is some evidence for disordered immunity in this disease. In our series there continues to be a preponderance, becoming significant, of HLA-A2. More patients are needed.

Finally we are assaying HLA antigens in patients and family members with affective disorders, eg. manic-depressive psychoses, since it is known that forms of this disease are genetic. We are looking for linkage between HLA and this disease.

Significance for Cancer Research and the Program of the Institute:
Cell surface molecules are involved in important recognition phenomena controlling normal growth and development. Malignancies arise when this recognition mechanism fails. Thus the study of these molecules is of critical importance to understanding normal growth and development and malignancy. Fortunately many cell membrane structures are antigenic, allowing identification by standard immunologic techniques. It is our purpose to study selected plasma membrane antigens and their respective antibodies.

Neuraminidase treated tumor vaccines are being widely used in human and animal tumor immunotherapy. An understanding of the immunological nature of the cell surface antigenic change following neuraminidase treatment is essential to the rational application of this promising mode of therapy. Moreover, the study of the natural autoantibodies to the neoantigen may be of great importance in understanding natural cell death or mechanisms of immune surveillance.

The cell surface histocompatibility antigens are a product of an extremely polymorphic genetic system which must have important survival value for the individual irrespective of its role in allograft rejection. For example, the possession of a given HLA specificity may confer, by mechanisms unknown, susceptibility (or resistance) to a particular disease. Therefore, one rational approach to assessing the natural biological significance of HLA antigen is the study of the association of disease with these antigens. If such associations are found, they have double significance. Closer medical observation of individuals possessing disease-associated HLA antigens may result in earlier diagnosis and better control of the disease. In addition, such knowledge may help us design experiments to probe the disease's basic pathophysiology.

Proposed Course of Project: Further studies of the antigen revealed by neuraminidase treatment of human lymphocytes will focus on the function of the antibody, the tissue distribution of the antigen and the biological significance of this phenomenon. A variety of patients with diseases, especially malignancies and disorders of immunity, will be typed for HLA antigens. In particular, we will continue large scale prospective studies of the association of HLA antigens with survival in acute leukemia, lung cancer, and melanoma. Correlations will be sought, and experiments designed to probe the pathogenesis of the disease will be attempted.

Publications:

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Rogentine, G. N.: Studies of the naturally occurring human antibody to neuraminidase treated human lymphocytes. Antibody levels in normal subjects, cancer patients and immunodeficiency patients. J. Natl. Cancer Inst. 54: 1307-1311, 1975.

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Tarpley, J. L., Chretien, P. B., Rogentine, G. N., Jr., Twomey, P. L., and Dellon, A. L.: Histocompatibility antigens and solid malignant neoplasms. Arch. Surg. 110: 269-271, 1975.

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Strober, W., Falchuk, Z. M., Rogentine, G. N., Jr., Nelson, D. and Kleaveman, H. L.: The pathogenesis of gluten-sensitive enteropathy. Ann. Int. Med. 83: 242-256, 1975.

Whisnant, J. K., Rogentine, G. N., Gralnick, M. A., Schlesselman, J. J., and Robbins, J. B.: Host factors and antibody response in hemophilus influenzae type B meningitis and epiglotitis. J. Infect. Dis., in press.

Whitaker, J. N., Herrmann, K. L., Rogintine, G. N., Stein, S. F., and Kollins, L. L.: Immunogenetic analysis and serum viral antibody titers in multiple schlerosis. Arch. Neurol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD-5018(c) 05 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Killer Lymphocyte Damage to Target Cell Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. A. Henkart, Ph.D.	Senior Investigator	IB, NCI
OTHER:	R. B. Blumenthal, Ph.D.	Senior Investigator	LTB, NCI
	J. N. Weinstein, M.D., Ph.D.	Expert/Consultant	LTB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

As previously described, we have established a model system to study the damage to foreign cell membranes which occurs when killer lymphocytes attack them. Antibody coated lipid bilayers serve as the target membranes for attack by normal human lymphocytes. Electrical measurements show that the conductance, and hence ionic permeability increases by up to several orders of magnitude when lymphocytes are added under conditions which allow killing. In order to define the nature of the lesions inflicted by the attacking lymphocyte, the ionic selectivity of membranes whose conductance was increased 20-100x was studied. This was accomplished by increasing the concentration of various salts on one side of the membrane and observing the membrane potential. No potentials were induced by a series of salts whose cations ranged as large as tetraethyl-ammonium and whose anions ranged as large as cacodylate. Thus the lymphocytes alter the lipid bilayer conductance in a manner very different from previously studied ionophores, and it was concluded that the lesions induced by killer lymphocytes are large enough to allow all such ions to pass freely through the membrane. Non electrical methods must be developed in order to study the size of the presumed channels.

Project Description:

Objectives: Our overall objective is to define the mechanisms by which lymphocytes destroy foreign cells. To this end we have directed the killing process into discrete steps which can be studied independently. In this project we have concentrated on the membrane lesion induced by lymphocytes in the target cell.

Methods Employed: A horizontal planar lipid bilayer is formed between two aqueous compartments by a modification of the Muller-Rudin technique. The lipids used are a mixture of oxidized cholesterol and DNP-phosphatidyl-ethanolamine; anti-TNP antibodies are used to form antibody coated lipid bilayers which are recognized by lymphocytes in a manner analogous to antibody coated target cells. Electrical conductivity measurements of the lipid bilayer give a measure of the ionic permeability.

Major Findings: As previously described, we have established a model system to study the damage to foreign cell membranes which occurs when killer lymphocytes attack them. Antibody coated lipid bilayers serve as the target membranes for attack by normal human lymphocytes. Electrical measurements show that the conductance and hence ionic permeability increases by up to several orders of magnitude when lymphocytes are added under conditions which allow killing. In order to define the nature of the lesions inflicted by the attacking lymphocyte, the ionic selectivity of membranes whose conductance was increased 20-100x was studied. This was accomplished by increasing the concentration of various salts on one side of the membrane and observing the membrane potential. No potentials were induced by a series of salts whose cations ranged as large as tetraethylammonium and whose anions ranged as large as cacodylate. Thus the lymphocytes alter the lipid bilayer conductance in a manner very different from previously studied ionophores, and it was concluded that the lesions induced by killer lymphocytes are large enough to allow all such ions to pass freely through the membrane. Non electrical methods must be developed in order to study the size of the presumed channels.

Proposed Course of the Project: Preliminary experiments have been carried out to use erythrocyte ghosts to determine the size of the membrane lesions. Ghosts have been resealed in the presence of ^{3}H-inulin to use intracellular labels as marker molecules of known size. A combination of two sizes of marker molecules will be used with double labelling techniques to determine the relative efflux rates of the markers.

Intracellular electrical recordings of target cells undergoing attack by killer lymphocytes, both T cells and antibody dependent K cells, will be attempted to compare the events seen in the lipid bilayer model with those occurring in a real target cell being destroyed. For comparison the destruction of cells by antibody and complement as well as membrane active drugs will be studied. These experiments should enable us to learn more about the final phases of target cell lysis after the membrane lesions have been inflicted on the target cell.

Significance for Cancer Research and the Program of the Institute: The process of lymphocyte destruction of foreign cells may be one of the most important mechanisms for the immunological rejection of allografts and tumors in vivo. Studies such as ours of the details of this process can be carried out in vitro and studied at the cellular and molecular level. Such studies are needed to complete our basic knowledge of the details of this important immunological defense process. Our studies have clearly shown that the target cell membrane is probably the primary target of killer lymphocyte attack, and the present studies have enabled us to clarify the nature of the membrane lesion by rigorous methods.

Publications:

Henkart, P. and Blumenthal, R.: The interaction of lymphocytes with lipid bilayer membranes: A model for the lymphocyte-mediated lysis of target cells (membrane permeability/ion channels). Proc. Natl. Acad. Sci. 72: 2789-2783, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5021 (c)-04 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Antigens Determined by the Major Histocompatibility Locus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
OTHER:	T. H. Hansen, Ph.D.	Investigator	IB NCI
	N. Shinohara, M.D.	Visiting Fellow	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being directed towards an understanding of the role of the products of the major histocompatibility complex in the cellular interactions of the immune response. Projects can be divided into three major categories: 1) Characterization of Major Histocompatibility Antigens: Complex resistant strains of mice have been reciprocally immunized to produce antisera detecting the products of limited regions of the MHC. Antigens determined by genes in the Ir region termed Ia antigens have been detected and have been shown to provide a major source of stimulation of the MLC reaction; 2) Characterization of the Response to Major Histocompatibility Antigens: The effect of genetic background on the ability to respond to particular Ia and H-2 antigens is being assessed; and 3) Characterization of the Receptor Site for Histocompatibility Antigens: Anti-idiotypic antibodies are being produced and their effects on in vitro and in vivo parameters of graft rejection are being assessed.

Project Description:

Objectives: 1) To produce antibodies against defined regions of the major histocompatibility complex. 2) To characterize the reactions of these antibodies with the cell surface of lymphocytes, and to determine the nature of the cells bearing individual antigens. 3) To attempt to produce anti-idiotypic antibodies against the receptors on these antibodies which detect cell surface histocompatibility antigens.

Methods Employed: Congenic resistant strains of mice differing only at their major histocompatibility loci have been reciprocally immunized in order to produce antibodies of known, restricted specificity. The antibodies have been characterized by assays of complement-mediated lymphocytotoxicity and by fluorescence microscopy using a fluoresceinated rabbit antimouse immunoglobulin as a developing agent.

The presence of unexpected specificities in these sera reacting with subpopulations of lymphoid cells has led to the development of methods of distinguishing subpopulation reactions. Additivity studies for cytotoxicity reactions and fluorescent staining have been performed with these reagents in combination with reagents of known specificity for B or T subpopulations of lymphoid cells. Also, reactions with fractionated lymphoid cell populations have been performed. T-cell derived tumors have been used for the absorption of the congenic antisera in order to reveal further specificities for antigens expressed preferentially on B cells. In addition, use has been made of congenic resistant animals bearing recombinant H-2 alleles in order to determine the genetic localization of many of the new antigens.

Antibodies against individual antigenic specificities have been isolated by adsorption onto lymphoid cells bearing those specificities, and the antibody-coated cells have then been injected back into syngeneic animals in order to immunize against the "receptor" portions of the antibody molecules. Sera have been collected from these animals after multiple immunizations and are being characterized for the presence of antibodies capable of reacting with the appropriate antihistocompatibility antigen receptors of antibodies and cells. Tests include inhibition of cytotoxicity, binding to solid-phase immunoabsorbents, passive hemagglutination, and *in vitro* and *in vivo* blocking of the immune response to tissue antigens.

Similar protocols are also being applied to antibodies raised in inbred rats differing at multiple histocompatibility loci, and to cross-species systems.

Major Findings: 1) Several of the antisera produced in congenic resistant mice have been shown to contain specificities additional to those expected in these strain combinations. These specificities have been shown to be determined by genes mapping within the Ir region of the murine major histocompatibility locus, and to be expressed preferentially on B cells.

Similar antigenic specificities have simultaneously been described by other laboratories, and the name Ia (Ir associated) antigens have been proposed to describe this new class of cell-surface antigens. Anti-Ia antibodies have been demonstrated in several H-2 antisera by absorption with T cell tumors.

2) The discovery of one of these Ia specificities on the B cells of a congenic recombinant mouse differing from another mouse only in the Ir region has been found to correlate with the previously described "one-way" mixed lymphocyte culture (MLC) reaction in this strain combination. This has led to the hypothesis that these Ia antigens may be associated with or responsible for the MLC reaction. This hypothesis has been confirmed experimentally using genetically defined MLC combinations and separated cells. Antibodies to distinct Ia antigens have been shown to specifically inhibit the MLC reaction at the level of apparent block of the stimulator cell.

3) Anti-Ia antibodies have been found to specifically block the Fc receptor of B lymphocytes. In addition one other genetic locus not linked to H-2 has been found to determine antigen(s) associated with the Fc receptor.

4) Anti-idiotypic reactions against the anti-H-2 antibodies have so far been demonstrable only in the presence of anti-allotype reactions and have been extremely weak.

5) An antibody response to a previously unrecognized H-2 antigen has been found and has been determined to be genetically controlled by a non-H-2 linked locus.

Significance to Biomedical Research and the Program of the Institute:
1) The Ia antigens appear to provide a new cell-surface marker for the B subpopulation of lymphocytes. In addition, the localization of the genes determining these antigens to the Ir region may indicate that these B cell surface structures are involved in the T cell-B cell collaboration necessary for many immune responses. If so, these antigenis may provide markers for dissecting the mechanism of that collaboration.

2) The specificity of the receptor for histocompatibility antigens should reside in the variable portion of the heavy and light chains of the relevant antibody molecules. Thus, anti-idiotypic antibodies against such receptors might be expected to distinguish those cells capable of reacting against individual histocompatibility antigens. Such antibodies, if obtained, could thus provide a new approach to modification of the immune response to cell surface antigens.

3) Understanding of the genetic control of response to H-2 antigens may make possible the modification of these responses in order to produce tolerance.

Proposed Course of Project: 1) Other antisera raised between congenic resistant strains of mice differing by known regions of the major histocompatibility complex will be screened for additional Ia specificities. The cellular distribution of these specificities will be determined (i.e., T cells vs B cells), and lymphoid tumors of known cellular origin will be used to absorb differential activities.

2) Models for the functional properties of these antigens which may explain their genetic localization to the Ir region will be further explored. These will include MLC reactivities, cellular cooperation models and cellular cytotoxicity models.

3) The relationship between Ia antigens and the Fc receptor will be pursued. Other non-H-2 antigens also associated with the Fc receptor will be investigated, as well as the Fc receptor of other cell populations such as macrophages and T cells.

4) Attempts at immunization against the receptors of anti-H-2 antibodies and cellular receptors for alloantigens will be carried out. Xenogenic immunization will be attempted.

5) Extensive testing as outlined above will be required in order to determine whether or not anti-idiotypic antibodies have been produced in any of our immunization protocols. If we can demonstrate the presence of such antibodies serologically, we will then attempt to purify them by selective immunoabsorption on columns bearing the appropriate immunoglobulins. The effects of these antibodies on in vivo cellular and humoral responses to tissue grafts will then be tested.

Publications:

Fathman, C.G., Cone, J.L., Sharrow, S.O., Tyrer, H. and Sachs, D.H.: Ia alloantigens detected on thymocytes using a fluorescence activated cell sorter. J. Immunol. 115: 584-589, 1975.

Dickler, H.B., Cone, J.L., Kubicek, M.T. and Sachs, D.H.: Detection of non-H-2 antigen(s) which, like Ia antigens, are associated with the Fc receptor of B lymphocytes. J. Exp. Med. 142: 796-801, 1975.

Arbeit, R.D., Sachs, D.H., Amos, D.B. and Dickler, H.B.: Human lymphocyte alloantigen(s) similar to murine Ir region associated (Ia) antigens. J. Immunol. 115: 1173-1175, 1975.

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Fathman, C.G. and Sachs, D.H.: Genetic control of the immune response to nuclease II. Detection of idiotypic determinants by the inhibition of antibody mediated nuclease inactivation. J. Immunol., in press.

Sachs, D.H., David, C.S., Shreffler, D.C., Nathenson, S.G. and McDevitt, H.O.: Ir Associated Antigens: Summary of the Proceedings of A Workshop on Ia Antigens Held November 21 and 22, 1974, at NIH, Bethesda, Maryland. Immunogenetics 2: 301-312, 1975.

Schmitt-Verhulst, A.-M., Sachs, D.H. and Shearer, G.M.: Cell mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Confirmation of genetic control of response to TNP-modified H-2 antigens by the use of anti-H-2 and anti-Ia antibodies. J. Exp. Med. 143: 211-217, 1975.

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Sachs, D.H., Schwartz, R.H. and Fathman, C.G.: The role of Ia antigens in MLC stimulation. In Proceedings of the Conference on the Role of Products of the Histocompatibility Gene Complex in Immune Responses Held at Brook Lodge, November 3-7, 1975. In press.

Sachs, D.H.: The Ia Antigens. In Inman, F.P. (Ed.): Contemporary Topics in Molecular Immunology. New York, Plenum Publishing Company, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5023 (c)-04 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Transplantation Antigens of Swine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
OTHER:	S. A. Rosenberg, M.D.	Chief, Surgery Branch	SURG NCI
	G. S. Leight, M.D.	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

NIH Animal Center, Poolesville, Maryland

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	.25

SUMMARY OF WORK (200 words or less - underline keywords)

A breeding program has been carried out starting with two miniature pigs from different sources and selecting offspring according to tissue typing procedures aimed at defining the major histocompatibility complex of this species. By this procedure three herds of miniature swine, each homozygous for a different set of histocompatibility antigens at the MHC have been developed. MLC reactions, serologic typing, and skin graft survival times all indicate that these animals are homozygous for a major histocompatibility locus analogous to HL-A of man or H-2 of the mouse.

Current projects include assessment of survival of organs and tissue transplants among and between members of these herds as a model for tissue typing and transplantation, purification and characterization of the major histocompatibility antigens of this species, and assessment of the immunologic parameters involved in tolerance to liver allografts in this species. Inbreeding in brother-sister fashion within each of these herds will be continued with the ultimate goal of establishing truly inbred animals if possible.

PHS-6040
(12-75)

Project Description:

Objectives: To develop at least two strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (SL-A). The animals will subsequently be used for *in vivo* experiments in organ and tissue transplantation and as a source of large numbers of cells from which cell surface antigens can be isolated, purified, and characterized chemically.

Methods Employed: Unlike classical inbreeding schemes which require about twenty generations to produce homozygosity, the approach being used with these swine involves selective breeding on the basis of histocompatibility typing of parents and offspring.

Four males and four females were purchased from commercial sources, and mating pairs were selected to be as varied as possible in order to assure the selection of different histocompatibility genes. Skin grafts were exchanged between the members of each pair and sera were obtained from the animals two weeks after the rejection of the grafts. The sera were tested for cytotoxic antibodies by lymphocytotoxic typing, and pairs which produced strong cytotoxic antibodies were bred.

Offspring from each breeding were tested serologically to determine which histocompatibility antigens had been inherited from the parents, and offspring which could be shown to possess the same antigen combinations were selected for further breeding. This process has now been repeated for four consecutive generations.

Major Findings: 1) Naturally occurring anti-red cell antibodies of swine are cytotoxic to lymphocytes (unlike their human counterparts) and must be absorbed from sera with packed red cells before leukocyte typing is performed.

2) Skin grafts between appropriate third and fourth generation animals were rejected in 8-14 days, and the rejection was accompanied by the production of high-titered lymphocytotoxic antibodies. Boosts with lymphocytes have been carried out in several related combinations and have yielded large quantities of high-titered antiserum.

3) Breeding of further generations has continued to be successful.

4) Typing of the fourth generation offspring yielded three breeding pairs of animals homozygous for serologically determined transplantation antigens. We therefore now have the capacity to breed three different herds of swine, each homozygous for a different SL-A antigen.

5) MLC assays of parents and offspring have continued to show a close correlation between this parameter of histocompatibility and the serologically determined transplantation antigens. Since this type of correlation has

previously been observed in studies of man (HL-A), monkey (RhL-A), mouse (H-2), rat (AgB) and dog (DL-A), this finding indicates that we are selectively breeding for homozygosity at a major histocompatibility complex (MHC) homologous to that of other mammalian species.

6) Skin graft survival has been found to be prolonged within each of the homozygous herds.

Significance to Biomedical Research and the Program of the Institute: One of the major problems in the study of cell surface antigens of human beings is lack of control of genetic constitution. The use of mice and rats as experimental models avoids this problem, but creates two new ones: 1) physiologically and anatomically these animals are often so different from human beings as to make comparisons and applications of findings difficult. 2) The size of these rodents makes it extremely difficult to obtain sufficient cells and tissues to permit quantitative chemical characterization of relevant cell surface antigens.

For both of these reasons it would be desirable to have animals of size comparable to human beings, whose genetic constitution with respect to histocompatibility could be controlled. The miniature pig, which attains an adult weight of about 200 lbs, is ideal for this purpose, and to date the experimental breeding pattern outlined above appears to be working well.

Proposed Course of Project: The breeding plan and typing will be continued. Lines will be established and separated, so that herds of any required size in each line can be produced.

Experiments in collaboration with the Surgery Branch will be continued in order to: 1) characterize the immune response to transplantation of skin, kidney, and liver in pigs across defined histocompatibility differences, and 2) to determine the basis of liver allograft tolerance which has previously been reported in pigs.

Studies of the SL-A antigens will be initiated at the biochemical level. These will center first on isolation and purification, and then on chemical and immunologic characterization.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5033(c)-04 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunotherapy of Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. D. Terry, M.D.	Assoc. Dir. for Immunology	IB NCI
OTHER:	R. J. Hodes, M.D.	Head, Immunotherapy Section	IB NCI
	S. A. Rosenberg, M.D.	Chief, Surgery Branch	SB NCI
	R. I. Fisher, M.D.	Senior Investigator	MB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

A controlled, randomized trial comparing immunotherapy to chemotherapy in stage I and stage II malignant melanoma has been initiated. A total of 46 patients have entered the trial, but it is too early to evaluate the results.

PHS-6040

Project Description:

Objectives: This project is designed to study various approaches to the immunotherapy of human malignancies.

Methods Employed: A new protocol (designated IB-2) was initiated in August, 1975. This protocol will evaluate the effect of 2 types of immunotherapy or 1 type of chemotherapy on the remission duration and survival of patients with stage I (level 4 or 5) and stage II malignant melanoma. Patients are stratified by stage, site of primary, and for stage II patients, presence or absence of clinically palpable lymph nodes and the number of histologically positive lymph nodes. Following stratification they are randomized to receive no further therapy (this is standard treatment for these patients) or chemotherapy with methyl CCNU or immunotherapy with BCG, or immunotherapy with BCG plus a vaccine that consists of three tissue culture grown allogeneic melanoma cell lines. These cell lines were a gift from Dr. Donald Morton, UCLA, and are grown in PPLO free conditions at Litton-Bionetics under the supervision of Dr. Edwin Matthews. Cells are treated with the enzyme neuraminidase to remove sialic acid (and thus render them more immunogenic) and frozen until ready for use.

All patients are worked up by the Immunology Branch or the Surgery Branch. Following randomization, they are assigned to the Medicine Branch for chemotherapy, the Immunology Branch for immunotherapy and the Surgery Branch for follow-up if there is no further treatment.

Bloods are drawn during pre-randomization work-up and subsequent to randomization and assays of cell mediated and humoral immunity are performed.

Major Findings: As of April 1, 1976, 46 patients have been randomized to this protocol. At a rate of 6 to 7 patients accrued per month, we will have entered 70 to 85 patients by the end of the 1st year. Since we estimate that we will need a total of 150 to 200 patients in order to have an evaluable trial, we should be able to close entry by August 1978. The state of the trial is summarized in the table:

	Control	McCCNU	BCG	BCG & Vaccine
Total Patients Entered	10	12	11	13
Recurrences	2	1	2	2
Deaths	0	0	0	0

In vitro assays have only been carried to the point of indicating that patients are being effectively immunized with the vaccine (see 5016). In addition, PPD tests indicate that all patients receiving BCG have converted to a positive skin test.

Significance for Biomedical Research and the Program of the Institute: Immunotherapy studies will explore the clinical effectiveness of manipulating the immune system in patients with cancer, and will also provide new information about the biology of the tumor-host relationship.

Proposed Course of Project: The IB-2 protocol will be continued.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5034 (c)-03 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization and Isolation of Human Lymphocyte Subpopulations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Dickler, M.D.	Senior Investigator	IB NCI
OTHER:	R. D. Arbeit, M.D.	Clinical Associate	IB NCI
	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.62	.62	

SUMMARY OF WORK (200 words or less - underline keywords)

The long term goal is to characterize and isolate human lymphocyte subpopulations and evaluate the function of these populations in the immune response of both normal and diseased individuals. Recent work under this project has shown that anti-HL-A alloantisera contain antibodies against antigens which are found primarily on B lymphocytes and which are associated with B lymphocyte Fc receptors. These "Ia-like" antigens may be important in immunoregulation.

Project Description:

Objectives: To characterize and isolate human lymphocyte subpopulations and evaluate the function of such populations in the immune response in both normal and diseased individuals.

Methods Employed: Lymphocytes from normal human donors were reacted with alloantisera and then evaluated for the presence of bound antibody and function of Fc receptors by fluorescent techniques. Alloantisera were fractioned by absorbtion with and elution from platelets.

Major Findings: Certain anti-HL-A alloantisera contained antibodies which inhibited the binding of complexed Ig to B lymphocyte Fc receptors. After absorbtion with platelets the alloantisera lost HL-A reactivity but retained the ability to react with B lymphocytes and inhibit Fc receptor function. Conversely, the HL-A antibodies eluted from platelets reacted with all lymphocytes but did not inhibit binding to Fc receptors. These data suggest that human B lymphocytes bear "Ia (Immune Response gene associated) like" antigens and that these antigens are associated with B lymphocyte Fc receptors.

Significance to Biomedical Research and the Program of the Institute: Characterization and isolation of lymphocyte subpopulations is of fundamental importance for an understanding of the nature of the immune response.

By analogy to the murine system, identification of human alloantigens associated with Fc receptors and primarily expressed on B cells may be important for understanding immunoregulation and interactions between immunocompetent cells, and may help characterize the genes responsible for such regulation.

Proposed Course of Project: The above alloantibodies will be used to further characterize human "Ia-like" antigens. 1) Relationship to the antigens responsible for transplantation rejection via MLC studies. 2) Genetic mapping via family studies. 3) Presence of absence on other immunocompetent cell populations such as "third population" lymphocytes and monocytes.

Publications:

Dickler, H.B., Arbeit, R.D. and Sachs, D.H.: The association between Fc receptor and certain lymphocyte alloantigens. In Seligmann, M., Preud'homme, J.L. and Kourilsky, F.M. (Eds.): Membrane Receptors of Lymphocytes. Amsterdam, North-Holland Publishing Company, 1975, PP. 259-266.

Arbeit, R.D., Sachs, D.H., Amos, D.B. and Dickler, H.B.: Human lymphocyte alloantigen(s) similar to murine Ir region associated (Ia) antigens. J. Immunol. 115: 1173-1175, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5035 (c)-03 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization of the Lymphocyte Receptor for Complexed Immunoglobulin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Dickler, M.D.	Senior Investigator	IB NCI
OTHER:	R. D. Arbeit, M.D.	Clinical Associate	IB NCI
	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
	P. A. Henkart, Ph.D.	Senior Staff Fellow	IB NCI
	S. E. Cullen, Ph.D.	Guest Worker	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are to characterize lymphocyte Fc receptors immunochemically, genetically, and functionally. Recent findings indicate that the Fc receptors of B cells and T cells but not "third population" lymphocytes nor macrophages are associated with Ia antigens. In the case of the B cell, the Fc receptors and Ia antigens appear to be distinct molecules, and there is also a single non-H-2 locus which determines antigens associated with the Fc receptor. Antigen antibody complexes can interfere with the mitogenic responses of lymphocytes. These findings indicate that Fc receptors may play a key role in Immunoregulation.

Project Description:

Objectives: (1) To immunochemically characterize the lymphocyte receptor for complexed antibody; (2) to evaluate the genotypic and phenotypic expression of the receptor; (3) to evaluate the role of this receptor in the immune response.

Methods Employed: Heat-aggregated immunoglobulin and/or antigen-antibody complexes are fluorochrome or radioactive isotope labelled, and then allowed to interact with isolated lymphocytes or macrophages from both man and animals. Binding is assayed by U.V. and phase microscopy, the Fluorescence Activated Cell Sorter (FACS), or by isotope counting. The interaction is studied by manipulation of the conditions of interaction, and by immunologic and chemical modifications of both lymphocytes and complexes. Genetic studies are carried out utilizing inbred, congenic, and/or recombinant mouse strains. Functionally, the effect of Ig complexes on the humoral immune response (plaque-forming cell assays), and on mitogenesis are analyzed. Precipitation and gel electrophoresis techniques are used in attempts to isolate the receptor.

Major Findings: The Fc receptors of murine B lymphocytes are associated with Ia antigens (Ir gene associated), but appear to be distinct molecules in that capping most Fc receptors does not appear to alter the distribution of Ia antigens. Antigens determined by at least one non-H-2 locus are also associated with B cell Fc receptors.

A portion of T lymphocytes also have Fc receptors but these appear to be of either lower affinity or are present in fewer numbers per cell than the Fc receptors of B lymphocytes. They also appear to be associated with Ia antigens.

The Fc receptors of macrophages and "third population" lymphocytes do not appear to be associated with Ia antigens.

Binding of Fc receptor positive lymphocytes to Ag/Ab coated plates inhibits their response to all B cell mitogens as well as concanavalin A but not to PHA.

Significance to Biomedical Research and the Program of the Institute: The receptor for complexed antibody has been shown to play a key role in antibody-dependent cell-mediated cytotoxicity. This immunologic mechanism may play an important part in host response to tumors. The Fc receptor/Ia antigen patch is hypothesized to be important in regulation of the tumor immune response and collaborations between immunocompetent cells.

Proposed Course of Project: (1) Studies of the Ia-Fc relationship by capping of Ia antigens and other cell surface markers followed by evaluation of Fc receptors. (2) Isolation and characterization of the molecular

structure and genetic expression of Fc receptors. (3) Analysis of the requirements for alteration or aggregation of Ig which allow binding to Fc receptors. (4) Analysis of the role of Fc receptors in regulation of the immune response using various functional assays.

Publications:

Dickler, H.B., Arbeit, R.D. and Sachs, D.H.: The association between Fc receptor and certain lymphocyte alloantigens. In Seligmann, M., Preud' homme, J.L. and Kourilsky, F.M. (Eds.): Membrane Receptors of Lymphocytes. Amsterdam, The Netherlands, North-Holland Publishing Co., 1975, pp. 259-266.

Ryan, J.L., Arbeit, R.D., Dickler, H.B. and Henkart, P.A.: Fc receptor-mediated inhibition of B lymphocyte mitogeneses. In Seligmann, M., Preud' homme, J.L. and Kourilsky, F.M. (Eds.): Membrane Receptors of Lymphocytes. Amsterdam, The Netherlands, North-Holland Publishing Co., 1975, pp. 389-392.

Dickler, H.B., Cone, J.L., Kubicek, M.T. and Sachs, D.H.: Detection of non-H-2 antigen(s) which, like Ia antigens, are associated with the Fc receptor of B lymphocytes. J. Exp. Med. 142: 796-801, 1975.

Ryan, J.L., Arbeit, R.D., Dickler, H.B. and Henkart, P.A.: Inhibition of lymphocyte mitogenesis by immobilized antigen-antibody complexes. J. Exp. Med. 142: 814-826, 1975.

Dickler, H.B.: Lymphocyte binding of aggregated immunoglobulin. Scand. J. Immunol., in press.

Arbeit, R.D., Henkart, P.A. and Dickler, H.B.: Lymphocyte binding of heat-aggregated and antigen-complexed immunoglobulin. In Bloom, B. and David, J. (Eds.): In Vitro Methods in Cell Mediated Immunity, Vol. II. New York, Academic Press, 1976, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5036 (c)-03 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Relationship Between Ir Genes and Immunoglobulin Variable Region Genes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
OTHER:	D. S. Pisetsky, M.D.	Clinical Associate	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1	.25

SUMMARY OF WORK (200 words or less - underline keywords)

Antibodies directed against idiotypic determinants on anti-staphylococcal nuclease antibodies from different mouse strains have been produced in rats and can be detected by the inhibition of antibody-mediated inactivation of nuclease. These antiidiotypes have been shown to be specific for the combining site (variable regions) of anti-nuclease antibodies since extensive absorption with normal mouse immunoglobulins fails to diminish their activity, and since antibodies to other sites on the Ig molecule do not inhibit inactivation. As nuclease is an antigen, the response to which is under control of H-2 linked immune response genes, it was of interest to determine the relationship between the idiotype and other genetic loci. By screening a variety of strains and offspring from appropriate matings between strains for the presence of idiotypes and other markers it has been shown that idiotype expression is linked to the heavy chain allotype marker and not to the immune response gene locus. The relationship of the idiotype to the problem of T-cell recognition of antigen is being investigated.

Project Description:

Objectives: The antibody response to staphylococcal nuclease (NASE) is under genetic control by gene(s) located in the Ir region of the mouse major histocompatibility complex (H-2). Since the antibodies produced to certain regions of this antigen have been previously shown to demonstrate restricted heterogeneity, we wish to determine whether or not the relevant Ir gene determines which variable region immunoglobulin products are produced. If the Ir gene is found to exert quantitative but not qualitative control over idiotypic antibody production, then a search for a distinct "T cell receptor" for nuclease (i.e., other than known IgV regions) must be anticipated.

Methods Employed: 1) Antibodies to NASE have been prepared in groups of mice of a variety of strains (both high and low responders) differing in H-2 type or allotype or both. Certain of these have been purified by affinity chromatography. Inbred Lewis rats have been immunized with the purified anti-NASE antibodies obtained from immune ascites from two A/J mice and SJL mice after immunization with NASE. Immunoabsorptions with normal immunoglobulins from the same strains have been used in order to determine whether or not antibodies reactive with the variable region (i.e., anti-idiotypic antibodies) have been produced. 2) Anti-idiotypic reactions have been quantitated by the inhibition of antibody-mediated enzyme inhibition. Such reactivities have been screened against anti-NASE antibody populations from the other strains of mice in order to determine the possible genetic linkage of idiotype to allotype and/or H-2 type. 3) Backcross animals have been screened for antibody levels and for allotype and idiotype expression in order to determine linkage of idiotype to other genetic markers.

Major Findings: By two separate criteria, anti-idiotype antibodies have been obtained. 1) After exhaustive absorption with whole normal A/J ascites or repeated passages over affinity chromatography columns to which normal A/J ascites was bound, antibodies remained which reacted with A/J αNASE but not with B10.A antibody. Prior to absorption these rat antibodies contained precipitating antibodies to either type (A/J or C57BL10) of antibody but no precipitating antibodies remained following absorption. 2) The rat anti-A/J αNASE antibodies prior to and following absorption reacted with the combining site of anti-NASE antibodies as evidenced by their ability to inhibit the anti-NASE antibody inactivation of NASE in enzymatic assay.

Among (B10.A x A/J) x B10.A backcross animals the A/J αNASE idiotype was found to be linked ($p<.005$) to heavy chain allotype.

Significance to Biomedical Research and the Program of the Institute: The development of an anti-idiotypic reagent directed toward antibodies under the control of an H-2 linked Ir gene may provide insight to the mechanism of Ir gene function. It may indicate whether the specificity of T cell recognition of antigens depends on similar variable region gene products to those responsible for B cell or antibody specificity. It may also lead to possible methods of controlling Ir gene expression.

Proposed Course of Project: There is suggestive evidence from other workers that the T cell antigen receptor shares the same idiotype as an antibody directed against that antigen. To study such a role for the idiotype in the generation of antinuclease antibodies, the effect of the antiidiotype on the production of antinuclease antibodies will be investigated. As the stimulation as well as the abolition of antibody production are both possible expressions of antiidiotype interaction with immune cell receptors, studies will be designed to assess both. An alternate approach, the effect of antiidiotype antibodies on the generation in vitro of antigen specific T cell factors, will be explored. If these two approaches fail to provide evidence for the role of the idiotype on T cells, an attempt will be made to immunize with immune T cells rather than antibody in order to determine whether another "idiotype" can be distinguished for the T cell receptor.

The antiidiotype preparations currently available are heterogeneous with specificities directed against a variety of antinuclease antibodies. To define more precisely the specificities represented, antibodies against subregions of the nuclease molecule will be prepared and the interaction of the antiidiotype with these fractionated antibodies studied. It is hoped that these fractionated preparations will provide more sensitive reagents in the study of the genetic basis for variable regions of antinuclease antibodies as well as possible study of the detailed structure of the nuclease receptor on T cells.

If linkage of idiotype expression with H-2 type is established, we will move directly to studies of cell surface receptors on T cells and to manipulations of responsiveness by means of pretreatment with antiidiotypic reagents.

If linkage to allotype is established, this relationship will be fully characterized, since previous studies of such linkage have not employed antigens under Ir control. An attempt will then be made to immunize with immune T cells rather than antibody in order to determine whether another "idiotype" can be distinguished for the T cell receptor.

Publications:

Fathman, C.G. and Sachs, D.H.: Genetic control of the immune response to nuclease II. J. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5038 (c)-03 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cell-Mediated Immunity to TNP-Modified Syngeneic Lymphocyte Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. M. Shearer, Ph.D.	Senior Investigator	IB NCI
OTHER:	A.-M. Schmitt-Verhulst, Ph.D.	Expert	IB NCI
	T. G. Rehn, M.D.	Clinical Associate	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

Murine thymus-derived lymphocytes were sensitized in vitro to trinitrophenyl-(TNP-) modified autologous lymphocytes. Cytotoxic effector cells were generated which specifically lysed only ^{51}Cr-labelled spleen target cells modified with the same agent and shared H-2K and/or H-2D alleles with the cells of the sensitizing phase. Experiments using F_1 hybrid responding lymphocytes sensitized and assayed with TNP-modified parental cells indicated that the homology required for lympholysis was either between modified stimulating and modified target cells, or among responding, modified stimulating and modified target cells. Antisera directed against serologically defined region products of unmodified target cells inhibited lysis of TNP-modified target cells, whereas anti-Ia antibodies had no detectable effect on lysis. Such experiments indicate that the effector cells are directed against specificities associated with serologically-defined region products and that some TNP groups are sterically close to the H-2K and H-2D region antigens. Further immunogenetic studies indicated that cell-mediated lympholysis response potential to TNP-modified autologous components is controlled by H-2-linked Ir genes. These findings demonstrate bifunctional H-2-associated regulation of cellular immunity to modified autologous lymphocytes, and raise the possibility that a similar type of H-2-linked control of immunity may be relevant for surveillance against viral-associated neoplasia.

Project Description:

Objectives: The purpose of this project was to investigate the genetic and mechanistic aspects of *in vitro* induction of cell mediated immunity, i.e. cell mediated lympholysis (CML) of murine lymphocytes to syngeneic and allogeneic lymphoid cells (normal and neoplastic) modified with trinitrophenol (TNP). This study was designed to extablish: a) whether the immune reactivity obtained was specific for the haptenic moiety (TNP) or whether it involved reactivity to some TNP-modified cell surface protein(s); b) whether response potential in this model was associated with an immune response (Ir) gene, and if so to determine the linkage pattern; and c) how specific the reactivity is *vis a vis* the genetic and cellular components of the induction and effector phases.

Methods Employed: Lymphoid cells from a number of inbred and congenic resistant mouse strains were heavily TNP-modified by treatment with trinitrobenzene sulfonic acid. These modified cells as well as lymphoma cells were used as immunogens and as radioactively-labelled cytotoxic target cells for unmodified syngeneic and allogeneic responding lymphocytes in primary *in vitro* sensitization for either CML or MLR reactivity. Effector cells from the CML sensitization were assayed for cytotoxicity on the appropriate (identical and nonidentical to genotype of immunizing cell type) ^{51}Cr-labelled targets and tested for anti-TNP antibody by a TNP-coated sheep erythrocyte plaque assay.

Major Findings: The major findings made thus far are:

1) Cell mediated cytotoxicity to TNP-modified syngeneic spleen and tumor cells was obtained. By assaying the sensitized lymphocytes with TNP-modified cells from an unrelated strain, major reactivity to TNP was ruled out. The response appears to be directed either to a modified cellular protein or to the modified protein plus the TNP moiety.

2) Weak but significant mixed lymphocyte reactivity has been obtained using TNP-modified syngeneic spleen cells as the stimulating population.

3) The relevant cell surface proteins modified by TNP to be immunogenic are controlled by the K and D serological regions of the H-2 complex.

4) H-2 linked immune response genes mapping to the left of I-A control response potential to the newly formed antigenic specificities.

5) Additional studies aimed at further defining the specificity of the lymphocyte receptor involved indicate that the haptenic group and the autologous H-2 antigen are intimately associated.

Significance to Biomedical Research and the Program of the Institute: The project is of basic immunological interest in that it describes a new

murine major histocompatibility linked immunological phenomenon. The fact that the phenomenon itself involves immunological reactivity to modified self lymphocyte surface proteins raises the possibility that this is a type of autoimmunity which is H-2 linked. This observation could provide an initial model for investigating the immunological effects of autoimmune and viral-modified lymphocyte surface proteins. This model represents the first demonstration of major histocompatibility control of the formation of new cell bound modified self antigens and of the response potential to these new antigens. The mapping of the immune response gene(s) to the K serologically detectable region of H-2 raises the possibility that the genes controlling response potential for a particular immune function map in the same H-2 region as the structural genes determining the cell surface structures (antigens) which are associated with that immune function. The model has the potential to provide a working hypothesis for bifunctional major histocompatibility control of disease susceptibility in those examples of HL-A-associated diseases.

Proposed Course of Project: Studies will be performed to determine whether the protein(s) modified by TNP are immune response gene products and/or immune response-associated antigens. Attempts will be made to solubilize, isolate, and characterize the modified cell surface proteins. Once the model has been genetically, chemically, and immunologically characterized, attempts will be made to create "foreign immunogens" using syngeneic cells by modification with viruses and autoimmune complexes. Studies are also in progress to determine whether a similar immunological model can be demonstrated using human leukocytes from normal and autoimmune individuals, and to determine whether any correlation exists between the response potential of individuals and HL-A type.

Publications:

Shearer, G.M.: Recognition of chemically modified autologous cells: Importance of the murine major histocompatibility complex. In Rosenthal, A. (Ed.): Ninth Leukocyte Culture Conference. New York, Academic Press, 1975, pp. 21-41.

Shearer, G.M.: Major histocompatibility-linked genetic control of murine cell mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Transplant. Proc. VII, suppl. 1: 109-113, 1975.

Shearer, G.M., Lozner, E.C., Rehn, T.G. and Schmitt-Verhulst, A.-M.: Mixed lymphocyte reactivity and cell mediated lympholysis to trinitrophenyl-modified autologous lymphocytes in C57BL/10 congenic and B10.A recombinant mouse strains. J. Exp. Med. 141: 930-934, 1975.

Schmitt-Verhulst, A.-M. and Shearer, G.M.: Bifunctional major histocompatibility linked genetic regulation of cell mediated lympholysis to trinitrophenyl-modified autologous lymphocytes. J. Exp. Med. 142: 914-927, 1975.

Schmitt-Verhulst, A.-M., Sachs, D.H. and Shearer, G.M.: Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes: Confirmation of genetic control of response to TNP-modified H-2 antigens by the use of anti-H-2 and anti-Ia antibodies. J. Exp. Med. 143: 211-217, 1976.

Schmitt-Verhulst, A.-M. and Shearer, G.M.: H-2 linked bifunctional genetic control of cell-mediated lympholysis to TNP-modified autologous cell surfaces. In Eijsvoogel, V.O. (Ed.): Leukocyte Membrane Determinants Regulating Immune Reactivity. New York, Academic Press. In press.

Shearer, G.M., Schmitt-Verhulst, A.-M. and Rehn, T.G.: Bifunctional histocompatibility-linked regulation of cell-mediated lympholysis to modified autologous cell surface component. In Katz, D.H. and Benacerraf, B. (Eds.): Proceedings of "The Role of Products of the Histocompatibility Gene Complex in Immune Responses". New York, Academic Press, 1976, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5039 (c)-03 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Fractionation of Lymphoid Cells Using Nylon Wool Columns

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Hodes, M.D.	Head, Immunotherapy Section	IB NCI
OTHER:	R. H. Schwartz, Ph.D.	Investigator	LI NIAID
	G. M. Shearer, Ph.D.	Senior Investigator	IB NCI

COOPERATING UNITS (if any)

Barry S. Handwerger, M.D., Department of Medicine, School of Medicine, University of Minnesota, Minneapolis, Minnesota

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

SUMMARY OF WORK (200 words or less - underline keywords)

Using nylon column adherence to fractionate lymphoid populations, it has been demonstrated that T cell-enriched and B cell-enriched populations could be separated from murine lymphoid preparations. By this methodology, T and non-T populations synergizing in the in vitro generation of cell-mediated cytotoxicity have been isolated. In another application of the same techniques, data has been generated suggesting that some T cell as well as B cell populations are capable of acting as stimulating cells in mixed lymphocyte reactions.

Project Description:

Objectives: 1) To identify subpopulations of lymphocytes and evaluate their functional significance; 2) To investigate the nature of hormonal influence on the immune system, by specifically identifying hormone receptors on lymphoid cells.

Methods Employed: During the past year our laboratory has made a significant advance in the separation of mouse lymphoid cells. We have helped develop a simple, rapid, inexpensive and efficient technique for separating large numbers of mouse spleen and lymph node cells into two populations, one enriched for thymus-derived or T lymphocytes and the other enriched for bone marrow-derived or B lymphocytes. The procedure is an extension of a technique first described by Julius, Simpson and Herzenberg (Eur. J. Immunol. 3: 645, 1973). It involves passage of cells over nylon wool columns at 37°C in the presence of Eagle's medium containing 5% heat-inactivated fetal calf serum. The B cells preferentially adhere to the columns, whereas the T cells pass through. After washing the T cells through the columns, the B cells are removed by mechanical agitation. Preliminary experiments indicate that this technique can be extended to other species including humans (Handwerger, B.S. and Schwartz, R.H., Transplantation, 18: 544-548, 1974).

Major Findings:

1) As previously reported, synergy of spleen subpopulations in the in vitro generation of cell-mediated cytotoxicity to allogeneic cells (Hodes, R.J., et al., J. Exp. Med. 140: 1646-1659, 1974).

2) Extension of these findings by demonstrating that T-cell and non-T-cell populations in normal mouse spleen also synergize in generating cell-mediated cytotoxicity to syngeneic TNP-modified cells (1). This model may be relevant in the investigation of autoimmune and anti-viral immune responses.

3) The nature of the stimulating or sensitizing cell in mixed lymphocyte responses has been investigated. Results indicate that at least some T-cell populations, as well as B-cell-enriched populations appear capable of stimulating allogeneic cells in these systems (manuscript in preparation).

Significance to Biomedical Research and the Program of the Institute: The interaction of two subpopulations of mouse spleen initially described in the response to alloantigens has subsequently been shown to occur in the responses to TNP-modified syngeneic cells and to syngeneic tumor cells as well. This therefore appears to be a potentially important mechanism of the host response to tumor antigens and altered "self" antigens as well as to allografts.

Proposed Course of Project: Experimental work on the topics described above has largely been completed. The methodology of cell fractionation is being actively applied to other areas of investigation described under separate project titles.

Publications:

Hodes, R.J., Hathcock, K.S. and Shearer, G.M.: Synergy between subpopulations of normal mouse spleen cells in the in vitro generation of cell-mediated cytotoxicity specific for "modified self" antigens. J. Immunol. 115: 1122-1125, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5041(c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characteristics of Lymphoid Populations of AKR/J mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. M. Zatz, Ph.D. Senior Investigator IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	0

SUMMARY OF WORK (200 words or less - underline keywords)

These studies suggest that the leukemogenic process in AKR mice selectively affects subpopulations of PHA and Con A responsive lymphocytes in the periphery and in the thymus, but that there is no generalized major deficit of T cells before leukemia development. These differential changes in spleen and thymus cell reactivity to mitogens may reflect a differential susceptibility of PHA and Con A responsive cells to infection by murine leukemia viruses.

PHS-6040
(12-75)

Project Description

Objectives: The objectives of this project are to: 1) define the defect (if any) in the lymphoid system of AKR/J mice which predisposes this strain to its high spontaneous incidence of thymic lymphoma and lymphocytic leukemia; 2) to characterize the leukemic cell populations in an attempt to determine the target cell which becomes transformed by the Gross Murine Leukemia virus.

Methods Employed: AKR/J mice aged 2 months to a year were studied. Lymph nodes, spleen and thymus cells of young (2-3 month), old (6-9 month), preleukemic (thymoma, but no peripheral organ involvement), and leukemic animals were evaluated for their surface expression of the theta (Θ) AKR (Thy 1.1) antigen, and responsiveness to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). Tissues from both animals bearing spontaneous lymphomas, and from young AKR/J mice injected with leukemic cells, were studied.

Major Findings: 1. Θ Antigen. There appears to be little change in the percent Θ-positive cells, as assessed by ^{51}Cr release, in the lymphoid organs of mice aged 2 months to a year, either normal or preleukemic. However the spleens and lymph nodes of leukemic animals contain an increased proportion of Θ-positive cells; these cells are more susceptible to lysis by a Θ serum and absorb more activity from such sera, suggesting an increase in the number of surface Θ determinants. This increase may be due to an increased density of Θ and/or to the increased surface area of such cells. 2) Responsiveness to Mitogens. There is a gradual loss with age in the responsiveness of peripheral lymphoid organs to PHA, which parallels that seen in other strains. A disproportionately greater loss of CON A responsiveness is seen which is most marked in preleukemic and leukemic animals. This disproportionate decline in responsiveness to PHA and CON A results in a progressively greater PHA/CON A ratio as animals age and become leukemic. In the leukemic animals, the changes in mitogen responsiveness are striking and distinctly different in peripheral lymphoid tissues and in thymus. While the PHA and CON A responses of leukemic spleen cells are lower than those of age-matched controls, there is as much as a 50 fold increase in the PHA response of thymoma cells, while the CON A response is often below that of controls. 3) Passage Experiments. In order to determine whether the distinct mitogen patterns observed in the thymus and spleens of leukemic AKR/J mice are stable, these cells have been passed into healthy young AKR recipients. Preliminary results suggest that the mitogen responses of recipient spleen and thymus resemble those seen in the spontaneous leukemic animal. The above results suggest the possibilities that 1) mitogen responsiveness is a more sensitive marker than the Θ-antigen for following changes in subpopulations during leukemogenesis in AKR/J mice; 2) the CON A responsive population may be more readily inactivated by MuLV; 3) microenvironmental influence in spleen and thymus affect the state of differentiation of the transformed cell populations.

Significance to Biomedical Research and the Program of the Institute: The AKR/J mouse strain provides a valuable model for the study of acute lymphocytic leukemias of the T-cell variety; similar leukemias presenting with thymic lymphosarcomas, have been reported in man. It is hoped that these studies will provide some answers to the question of how and why thymic lymphomas arise; and the nature of the cell populations in which they arise. These studies should also provide more fundamental information about the state of differentiation, and functional activity, of transformed leukemic cells.

Proposed Course of the Project: One aspect of this project, i.e. host influences on the growth of leukemia cells, has been greatly expanded and will be described in a new project report. See "Resistance to Leukemia".

Publications:

Zatz, M. M. Lymphocyte populations of AKR/J mice III. Changes in the preleukemic state. J. Immunol. 115:1168. 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER NCI ZO1 CBD 5042(c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Antigen Induced Changes in Lymphocyte Traffic

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. M. Zatz, Ph.D. IPA Investigator IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of Bacillus-Calmette Guerin (BCG) administration upon lymphocyte traffic was studied in BALB/cJ mice. Intravenous or subcutaneous injection of BCG resulted in increased localization (trapping) of ^{51}Cr-labelled syngeneic lymphocytes in the spleen or draining nodes. BCG-induced splenic trapping was biphasic, occurring 1-2 days after BCG injection, and reappearing 3 weeks later. Trapping in draining lymph nodes persisted and increased during the 24 days of study. These results are in contrast to those obtained with SRBC, where trapping was evident only within the first few days of antigen injection.

Additional studies revealed that prior injection of BCG influenced the ensuing magnitude of SRBC-induced trapping. Intravenous administration of BCG suppressed SRBC-induced splenic trapping; both intravenous and subcutaneous injection of BCG enhanced trapping to SRBC in the draining lymph nodes.

These studies suggest that one mechanism for potentiation of immune responses by BCG may operate via BCG's effects upon regulation of lymphocyte traffic to the lymphoid tussues.

Project Description:

Objectives: The objective of this project is to determine the factors which influence lymphocyte traffic to the lymphoid organs following antigenic challenge. It is known that a variety of antigens, tumors, allografts, adjuvants, and GVH reaction can induce alterations in lymphocyte homing patterns at the site of antigenic stimulation (Zatz, J. Exp. Med. 134:224, 1971). Such exposure to antigen in vivo usually results in increased localization of cells, termed "trapping," in spleens, or lymph nodes, depending upon the route of antigen administration. Since it is known that BCG is an adjuvant, the question of whether trapping is subject to BCG induced regulator mechanisms was asked.

Methods Employed: Recipient animals of different ages were subjected to various protocols and doses of BCG, prior to challenge with SRBC. The magnitude of ^{51}Cr labelled lymphocyte migration to recipient lymphoid organs was then quantitated in BCG treated or control animals.

Major Findings: The effects of Bacillus-Calmette Guerin (BCG) administration upon lymphocyte traffic was studied in BALB/cJ mice. Intravenous or subcutaneous injection of BCG resulted in increased localization (trapping) of ^{51}Cr-labelled syngeneic lymphocytes in the spleen or draining nodes. BCG-induced splenic trapping was biphasic, occurring 1-2 days after BCG injection, and reappearing 3 weeks later. Trapping in draining lymph nodes persisted and increased during the 24 days of study. These results are in contrast to those obtained with SRBC, where trapping was evident only within the first few days of antigen injection.

Additional studies revealed that prior injection of BCG influenced the ensuing magnitude of SRBC-induced trapping. Intravenous administration of BCG suppressed SRBC-induced splenic trapping; both intravenous and subcutaneous injection of BCG enhanced trapping to SRBC in the draining lymph nodes.

These studies suggest that one mechanism for potentiation of immune responses by BCG may operate via BCG's effects upon regulation of lymphocyte traffic to the lymphoid tissues.

Significance to Biomedical Research and the Program of the Institute: This work is of importance in that it examines the effect, in vivo, of BCG, an agent clinically used for immunotherapy. The regulatory mechanisms described above may play an important role in the in vivo influence of BCG on tumor immunity.

Proposed Course of Project: Not under current investigation.

Publications:

Zatz, M.M. and Gershon, R.K.: Regulation of Lymphocyte Trapping: The Negative Trap. J. Immunol. 115: 450-453, 1975

Zatz, M.M.: Lymphocyte Trapping. Differential Effects of ATS and Irradiation of Trapping in Lymph Nodes and Spleen. Immunology (in press).

Zatz, M.M.: Effects of Cortisone on Lymphocyte Homing. Israeli J. Med. Sci. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5043 (c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

AKR Leukemic Lines With T and B Cell Characteristics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. Zatz, Ph.D.	IPA Investigator	IB, NCI
OTHER:	R. S. Greenberg, M.D.	Clinical Associate	IB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.125	.125	0

SUMMARY OF WORK (200 words or less - underline keywords)

Several unusual murine lymphoma lines were established and studied for antigenic and functional characteristics. The lines were derived from AKR/J strain mice and are noteworthy in that they exhibit characteristics of both T and B cells. When passaged into young normal AKR/J hosts the cells appear first like T-cell lymphomas and then differentiate into tumor cells with aspects of both T and B cells. Characteristics studied were Thy 1 antigen, Ia antigen, Fc receptor, complement receptor, mitogen responsiveness, and SIg. Factors controlling the expression of these markers in vivo and in vitro are being studied.

Project Description:

Objectives: The objective of this project is to investigate and characterize several unusual variant lines of AKR murine leukemias derived from spontaneous tumors arising in old mice which had been thymectomized at one month of age. This system was selected as a model for a rarely occurring natural variant of AKR leukemia in which the host animal has an atrophic thymus rather than the usual thymoma.

Methods Employed: Passage lines of AKR_5 tumors were established and continued by intravenous injection of 1 x 10^5 spleen cells into young AKR hosts. Donor cells were initially derived from tumors arising in old animals, which had been totally thymectomized at one month of age. These lines have been named AKTB cells.

The passage has been presently carried 15 passage generations during which cells were studied for various characteristics including theta antigen (by surface immunofluorescence and cytotoxicity), surface immunoglobulin (SIg), in vitro synthesis of immunoglobulin, and mitogen responsiveness. Fc receptor, complement receptor, Ia antigens, and aggregate receptors were also studied. Kinetic studies on the acquisition of the above markers were carried out, with preliminary attempts to determine the factors regulating their expression.

Major Findings: 1) Theta antigen is present on 90-100% of spleen and lymph node (LN) cells of AKTB tumor lines. Theta was best demonstrated using CBA-anti AKR anti-theta serum from hyperimmunized animals counterstained with fluorescein-labelled rabbit anti-mouse $gamma_2$ immunoglobulin.

2) SIg is demonstrable on up to 100% of AKTB spleen cells by the end of each passage. This SIg is of a single class--μ--as demonstrated by specific immunofluorescence, and does not react with anti-alpha chain or anti-gamma chain labelled antibody. Similar results were obtained using polyvalent anti-immunoglobulin.

3) Within each passage of the tumor line the percentage of SIg-positive spleen cells increases progressively from 0% (around 10 days) to 90-100% (around 24 days) at times when spleens are grossly enlarged (5-10 fold) and cells are theta-positive.

4) LN and thymus cells from tumor-bearing animals with 90% SIg-positive spleen cells and gross lymphadenopathy are theta-positive and SIg-negative.

5) Simultaneous staining for SIg and theta antigen reveal significant numbers (more than 50%) doubly marked cells in the spleen.

6) Immunoelectrophoresis and autoradiography studies on immunoglobulin synthesis in vitro by spleen cells from these cell lines demonstrate significant μ and k chain production in overnight cultures.

7) Mitogen studies revealed that the DNA synthesis of AKTB cells (late in passage, SIg-positive) was increased in response to LPS but was suppressed by PHA or Con-A. AKTB spleen cells early in passage (when cells were theta-positive and SIg negative) and AKTB cells in lymph nodes responded to PHA and Con-A but not to LPS.

8) Ia antigens and Fc receptors are present on AKTB spleen cells and their presence correlates with the presence of SIg on those cells. LN cells are Ia and Fc receptor negative.

9) SIg and theta antigen are regenerated on the surface of cells cultured in vitro after these markers are removed by capping with specific antisera. They also persist on tumor cells maintained in long-term culture.

Significance for Cancer Research and Program of the Institute: The results of these studies bear upon a number of issues of fundamental importance to both cellular immunology and also to viral leukemogenesis:

1) There are very few cell lines which bear clear markers for both T and B cells, although a normal subpopulation with such characteristics has been identified in small numbers. Perhaps this line represents an abnormal proliferation of this population or an arrested phase of normal lymphoid development, implying transitions between T and B cells.

2) Alternatively the SIg on these cells may represent an abnormal amount of the "normal" T cell immunoglobulin, so that this line might be a way to examine "IgT" and the putative T cell antigen receptor.

3) The difference between spleen and LN cells in the expression of SIg within the same animals suggests that this system may be very helpful in studying the problem of "microenvironment" on cell development within the lymphoid system.

4) The ability to isolate SIg from these cells may allow the generation of anti-idiotype antibody in another species, and thus answer the question of whether or not AKR leukemia is a monoclonal disease.

5) Cell-free extracts of these tumors may allow the identification of a second viral type in the murine leukemia virus (MLV) system.

6) Since the AKTB line is in some sense a B cell leukemia, it may provide a system in which to examine B cell differentiation.

Proposed Course of Project: Specific immediate plans to follow up some of the above suggestions include: passage studies of specific lymphoid elements (LN, spleen and thymus); in vitro culture lines: interactions among cell populations; studies with thymic factors and epithelium studies of old

non-leukemic animals for susceptible cell populations for transformation and proliferative characterization of the SIg.

Publications:

Greenberg, R. S., and Zatz, M. M.: Spontaneous AKR lymphoma with T-and B-cell characteristics. Nature 257: 314-316, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5045(c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cell-mediated Immunity to N-modified Syngeneic Lymphocyte Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. M. Shearer, Ph.D.	Senior Investigator	IB, NCI
OTHER:	T. G. Rehn, M.D.	Clinical Associate	IB, NCI
	A.-M. Schmitt-Verhulst, Ph.D.	Expert	IB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Splenic lymphocytes from C57Bl/10 congenic mouse strains were sensitized in vitro to N-(3-nitro-4-hydroxy-5-iodophenylacetyl)-β-alanylglycylglycyl-(N) modified autologous lymphocytes. The effector cells generated were assayed on a series of N-modified tumor cells. The results indicated in all cases that both N modification of the targets and H-2 homology between the modified stimulating and target cells are required for lysis to occur. In each case the effector cells were found to lyse N-modified target cells only when there was homology at either or both ends of the major histocompatibility complex (MHC) between the stimulator and target cells. B10.BR and B10.A effector cells lysed targets sharing K end H-2 serological regions greater than target cells sharing D-end serological regions. The C57BL/10 effector cells were shown to react to the K end greater than the D end, which differed from the equal reactivity seen in the TNP system for this strain. The data are consistent with the hypothesis that the antigen recognized by the effector cell includes an altered H-2 serological cell surface product. That the reaction is not "hapten specific" and the H-2 homology is required only for effector:target cell interaction was excluded by the use of two F_1 combinations in which lysis of only N-modified target cells sharing the H-2 haplotype with the stimulating parental strain was obtained.

Project Description:

Objectives: The purpose of this project was to investigate in vitro induction of cell-mediated immunity, i.e., cell-mediated lympholysis (CML) of murine lymphocytes to syngeneic and allogeneic lymphoid cells (normal and neoplastic) modified with N-(3-nitro-4 hydroxy-5 iodophenylacetyl)β-alanylglycylglycyl azide (NIP). This study was designed to establish: a) whether the immune reactivity obtained was specific for the haptenic moiety (NIP) or whether it involved reactivity to some NIP-modified cell surface protein(s); b) whether response potential in this model was associated with an immune response (Ir) gene, and if so to determine the linkage pattern; c) how specific the reactivity is vis a vis the genetic and cellular components of the induction and effector phases; d) if the immune reaction is composed exclusively of T cells or if B cells are also required; and e) to determine what effect minor alterations in the NIP molecule have on CML specificity.

Methods Employed: Similar to those outlined in NCI ZO1 CBD 5038, except the NIP hapten was used instead of TNP.

Major Findings: 1) Cell-mediated cytotoxicity to NIP-modified syngeneic spleen cells was obtained which was specific for NIP-modified K and D H-2 serological region gene products on lymphocyte surfaces. 2) Weak but significant mixed lymphocyte reactivity was obtained to NIP-modified syngeneic lymphocytes. 3) H-2-linked Ir genes appear to control cell-mediated immunity to the new antigenic determinants. 4) Specificity studies using NIP and TNP determinants with or without a tripeptide spacer inserted between the haptenic group and the cell surface suggest that the lymphocyte possesses a single receptor recognizing self (either modified or unaltered) plus at least part of the hapten.

Significance for Biomedical Research and the Program of the Institute: This project is of basic immunological interest in that it describes a new murine major histocompatibility-linked immunological phenomenon. The fact that the phenomenon itself involves immunological reactivity to modified self lymphocyte surface proteins raises the possibility that this is a type of autoimmunity which is H-2-linked. This observation could provide an initial model for investigating the immunological effects of autoimmune and viral-modified lymphocyte surface proteins. This model represents the first demonstration of major histocompatibility control of the formation of new cellbound modified self antigens and of the response potential to these new antigens. It has the potential to provide a working model for bifunctional major histocompatibility control of disease susceptibility in those examples of HL-A-associated disease.

Proposed Course of Project: Studies will be performed to determine whether the protein(s) modified by NIP are immune response gene products and/or immune response-associated antigens. Attempts will be made to

solubilize, isolate, and characterize the modified cell surface proteins. Once the model has been genetically, chemically, and immunologically characterized, attempts will be made to create "foreign immunogens" using syngeneic cells by modification with viruses and autoimmune complexes. Studies are also in progress to determine whether a similar immunological model can be demonstrated using human leukocytes from normal and autoimmune individuals, and to determine whether any correlation exists between the response potential of individuals and HL-A type.

Publications:

Rehn, T.G., Shearer, G.M., Koren, H.S. and Inman, J.K.: Cell-mediated lympholysis of N-(3-nitro-4-hydroxy-5-iodophenylacetyl)-β-alanyl-glycyl-glycyl-modified autologous lymphocytes: Effector cell specificity to modified cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. J. Exp. Med. 143: 127-142, 1976.

Shearer, G.M., Rehn, T.G. and Schmitt-Verhulst, A.-M.: Role of the murine major histocompatibility complex in the induction of cell-mediated lympholysis to chemically modified autologous lymphocytes. Transplant. Rev. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5047 (c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Serological and Chemical Analysis of Ia Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Cullen, Ph.D.	Guest Worker	IB NCI
OTHER:	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
	B. D. Schwartz, M.D.	Research Associate	LI NIAID

COOPERATING UNITS (if any)

Chella S. David, Ph.D., Department of Genetics, Washington University School of Medicine, St. Louis, Missouri

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.62	.62	

SUMMARY OF WORK (200 words or less - underline keywords)

The Ia alloantigens are the only isolated gene products of the immune response control region (I region) of the mouse major histocompatibility complex (MHC). The molecules bearing Ia alloantigenic determinants have been directly implicated in the control of immune response function by experiments showing that anti-Ia alloantisera inhibit such functions. Since Ia antigens may have an active role in the control of the immune response, these molecules must be analyzed to see if their structural features can explain how they carry out their proposed function.

The research described here includes structural analysis and serological investigation of Ia molecules. Structural analysis includes localization of antigenic sites, and attempts to determine whether non-MHC genes determine parts of the Ia molecules. Serological investigations utilize immune precipitation by alloantisera as a screening tool for analysis of Ia polymorphism and as an auxiliary mapping technique to gather information about the genetic organization of the I region.

Project Description:

Objectives: The purpose of this project is to gain a better understanding of the genetic fine structure of the immune response (I) region of the mouse major histocompatibility complex (MHC), and to investigate some of its gene products.

Besides controlling several types of immune functions through the Ir genes, MLR genes and GVH genes, the I region determines the presence of a series of alloantigenic specificities on the surfaces of lymphoid cells. Recombination has separated some of these determinants, thus enabling a subdivision of the I region into three subregions some of which are mapping sites for several of the known Ia specificities. Solubilized Ia antigens which map in different subregions have been shown to be separable by indirect immune precipitation (Cullen et al., Proc. Nat. Acad. Sci. 71:648, 1974; Cullen and Nathenson in The Immune Response: Genes, Receptors, Signals, ed. Sercarz and Williamson, Academic Press, 1974, p. 191; Sachs et al., Transplantation, in press). We have also shown that antigenic specificities mapping in the same subregion are coprecipitable. Several areas of investigation are included in this project. (1) Newly described Ia antigenic specificities require examination to determine whether they are chemically similar to previously described specificities. In addition, the precipitation technique may be useful to predict the subregion in which the new specificities may map. Recombinants appropriate for mapping of the new specificities are so far unavailable. (2) The antigens should be further characterized chemically. (3) As a long-range goal, possible association between the Ia specificities and molecules mediating the I region immune functions could be investigated.

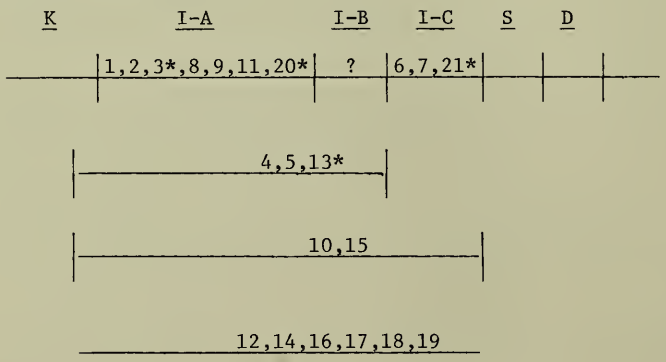
Methods Employed: Radiolabelled detergent solubilized antigen preparations made from mouse spleen cells are the materials analyzed. Antisera have been raised by Dr. David and Dr. Sachs. The specific antigen molecules are isolated by indirect immune precipitation and the components precipitated are analyzed by SDS-polyacrylamide gel electrophoresis. Other separation methods employing gel filtration or charge dependent separation (ion exchange chromatography, isoelectric focusing) may be employed. Affinity chromatography with plant lectins is being used to isolate glycoproteins.

A new and better method for indirect immune precipitation has been developed this year in collaboration with Dr. B. Schwartz. The radioactive antigen is complexed with alloantibody as usual, but instead of using an anti-globulin antibody to precipitate these complexes, formalinized, heat-killed Staphylococcus aureus is added. These bacteria bear on their surface protein A, which has specific affinity for IgG of most subclasses and which therefore specifically binds the antigen-antibody complexes. This new type of precipitation is faster, more efficient, and the radioactive Ia antigens subsequently eluted from the organisms can be subjected to electrophoresis with greater ease and less generation of artifacts (Cullen, S.E. and Schwartz, B.D., 1976, submitted).

Major Findings: Using precipitation analysis we have been able to show that certain antisera raised by Dr. David do indeed contain anti-Ia antibodies, which was suspected from serological testing. The Ia specificities 12, 13, and 14 were authenticated in this way (David, C.S., Colombani, J., Colombani, M. and Cullen, S.E., 1976, Transplantation, in press).

Using sequential precipitation analysis we have been able to show that in F_1 hybrid animals, the antigenic specificities determined by allelic segments of the I-A subregion of the I genetic region are found on independently precipitable molecules. The separability of parental haplotype specificities in the F_1 indicates that there is probably no production of "hybrid" molecules and that therefore there is no implication that polypeptide chains from different Ia molecules are mixed during assembly. It is also an indication that there is probably no detergent-stable association of non-homogeneous Ia molecules. This latter point is important for the interpretation of the coprecipitation of specificities which we observe in other experiments. (These data and others presented in the prior progress report are included in Cullen, S.E., David, C.S., Cone, J.L. and Sachs, D.H., J. Immunol. 116:549, 1976.)

We have been able to suggest the mapping position for genes determining several other previously unmapped Ia specificities by showing that they coprecipitate with specificities of known map position. The following genetic map shows the specificities to which we have assigned a probable map position (denoted by an asterisk).



In summary, we have shown that

1. WIa.20 (W is a workshop designation indicating a serological reactivity tentatively identified as belonging to the Ia series) coprecipitates

with Ia.8 and Ia.9, indicating that it is really an Ia specificity and that it may map in the I-A subregion.

2. WIa.21 coprecipitates with Ia.7 and is thus confirmed to be an Ia specificity and suggested to map in the I-C region. (Cullen, S.E., Sachs, D.H. and David, C.S., 1976. Proceedings of the 10th Leukocyte Culture Conference, in press.)

3. Ia.3 had been tentatively assigned to the I-B subregion, but it coprecipitates with Ia.8 and Ia.9, and thus is probably determined in the I-A subregion. This leaves the I-B subregion with no definitely assigned Ia specificity although it is the mapping site for several Ir genes.

We have also shown that Ia molecules are composed of two glycopolypeptide chains which have variable mobility on electrophoresis. The molecules shown to differ include:

a. Molecules from different subregions of the same haplotype.

b. Molecules from the "allelic forms" of same subregion of different haplotypes

c. Molecules from the same subregion from different strains with nominally shared haplotypes

d. Molecules from the same subregion and haplotype, but from different mouse strains with different genetic backgrounds (i.e. different non-MHC genes).

Attempts are being made to discover the reason for this variability. There may be chemical differences of a trivial or fundamental nature between the molecules. As noted in (d), the variation may be an effect mediated by genes elsewhere in the genome, and this would be a most unusual effect requiring further documentation and analysis. In one case tested, removal of carbohydrate chains by digestion with a mixture of glycosidases resulted in a shift in electrophoretic mobility, but relative mobilities of the two Ia peaks were unchanged.

Significance to Biomedical Research and the Program of the Institute: The study of the organization of the I genetic region and of the structure of its gene products has fundamental importance for a greater understanding of the control of the immune response. The immunologic mechanisms involved affect several areas of biomedical research including transplantation, immune deficiency disease, autoimmune disease, and of course, immunological approach to cancer treatment. A direct result of this study should be more detailed knowledge of the complexity of the I region, and this should enable finer distinctions to be made in correlating structures with function. If the Ia

antigens themselves are involved in the control of the immune response, greater knowledge of their chemical nature will be greatly needed.

Proposed Course of Project: We will continue to analyze the mapping of the I region by sequential precipitation analysis. Predictions of I subregion map position may be possible for specificities 4, 5, 12 and 13. We will also try to analyze the observed differences in electrophoretic mobility by using different gel porosities (to determine whether migration is anomalous by means of a Ferguson plot). We will try to determine whether alterations in carbohydrate structure are responsible for the differences. We will direct special attention to analysis of strain pairs which would shed more light on the possible effect of non-Ia genes (e.g. A/J and B10.A or C57BR and B10.BR).

Publications:

Cullen, S.E. and Schwartz, B.D.: An improved method for isolation of H-2 and Ia alloantigens using immune precipitation induced by protein-A bearing staphylococci. (Submitted)

Cullen, S.E., David, C.S., Cone, J. and Sachs, D.H.: Evidence for more than one Ia antigenic specificity on molecules determined by the I-A subregion of the mouse major histocompatibility complex. J. Immunol. 116: 549-553, 1976.

David, C.S., Colombani, J., Colombani, M. and Cullen, S.E.: Murine I region associated antigen system: Identification and chemical characterization of specificities Ia.12, 13, and 14. Transplantation, in press.

Cullen, S.E., Sachs, D.H. and David, C.S.: Use of immune precipitation to identify and suggest mapping position for new Ia specificities. Proceedings of the 10th Leukocyte Culture Conference, Academic Press, New York. In Press.

Schwartz, B.D. and Cullen, S.E.: The chemistry of mouse and guinea pig Ia antigens. Brook Lodge Symposium, in press.

Cullen, S.E., Freed, J.H. and Nathenson, S.G.: Structural and serological properties of murine Ia antigens. Transpl. Rev., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5048 (c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Attempted Isolation of Fc Receptor: Relationship to Ia Antigen Molecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Cullen, Ph.D.	Guest Worker	IB NCI
OTHER:	H. B. Dickler, M.D.	Senior Investigator	IB NCI
	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI
	P. A. Henkart, Ph.D.	Senior Staff Fellow	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	

SUMMARY OF WORK (200 words or less - underline keywords)

Isolation of Fc receptor from detergent solubilized radiolabelled lymphoid cells is being attempted. The affinity of Fc receptor for antigen-antibody complexes is being exploited as a means to selectively bind radiolabelled Fc receptor. Design of rigorous controls is being carried out. SDS polyacrylamide gel electrophoresis is used to analyze materials which are bound. The radiolabelled cell components which bind to antigen-antibody complexes do not appear to be similar to Ia antigens in molecular weight, although anti-Ia antisera are capable of blocking binding of fluoresceinated IgG aggregates to Fc receptor. The bound components do appear to be similar in molecular weight to molecules precipitated by another, relatively uncharacterized, alloantiserum which also blocks binding of fluoresceinated IgG aggregates to Fc receptor. Further correlation between these entities is being carried out.

Project Description:

Objectives: Anti-Ia antisera have been shown capable of blocking binding of fluoresceinated Ig aggregates to lymphocytes. This blocking of Fc receptor activity was specific for anti-Ia antibody and was not due to anti-H-2 antibody (Dickler, H.B. and Sachs, D.H., J. Exp. Med. 140:779, 1974). In view of these findings, it was deemed worthwhile to attempt isolation of Fc receptors to determine whether they were structurally associated with Ia antigen molecules or merely fortuitously close on the cell surface.

Methods Employed: Spleen cells were radiolabelled with ^{3}H-leucine, and the labelled cells were detergent solubilized. The detergent-soluble material was then used as a putative source of labelled Fc receptor.

Binding to immobilized IgG aggregates was found unsatisfactory due to nonspecific absorption problems. Binding to immune complexes formed between TNP-KLH and affinity purified anti TNP antibodies is now being tested.

Very preliminary data also raises the possibility that Fc receptors might be specifically isolated through the use of an alloantiserum which may detect allotypic variants.

Major Findings: The binding to immobilized IgG aggregates was pursued for some time and attempts were made to reduce the non-specific absorption effects. This technique was eventually abandoned however because it could not be rendered specific.

Preliminary results using immune precipitates show binding of material in the molecular weight range of 60-70,000 daltons and 15-20,000 daltons. These results are not yet sufficiently clear or reproducible however.

Other extremely preliminary data show that an alloantiserum capable of blocking binding of fluoresceinated IgG aggregates to Fc receptor bearing cells is also capable of precipitating material of the same molecular weight range from detergent solubilized radiolabelled cells.

Thus far the molecules absorbed to immune complexes or identified with this antiserum appear to be different from Ia in molecular weight. Experiments definitively identifying the molecules as authentic Fc receptors are still required. The relation of these molecules to Ia antigens remains to be elucidated.

Significance to Biomedical Research and the Program of the Institute: It is important to know whether the association noted between alloantigens and Fc receptor when tested at the cell surface is a reflection of some physical bond between these entities at the molecular level. This distinction is also of interest to help uncover the means by which the I genetic

region which determines Ia antigens exerts a major role in several immune response functions.

Proposed Course of Project: We will attempt to determine whether it is justified to specifically identify radiolabelled material binding to immune complexes as Fc receptors. The ideal approach would be to show that antigen-$F(ab')_2$ complexes do not bind the material (because they lack the Fc portion of the IgG molecule). Such complexes may not be precipitable however, and this control therefore may be impractical. An alternative control would be to use antigen-IgA complexes in a binding test. IgA is known to bind to Fc receptor, and binding to antigen-IgA complexes would be expected to be negative. This is a control which probably can be done with relative ease.

We also have plans to check the specificity of the alloantiserum which detects variation in a molecule which may be identifiable as Fc receptor. Genetic backcross testing will be done to determine whether the animals whose Fc receptors can be blocked by this antiserum are also bearers of the molecules which the antiserum has been found to precipitate. If these two traits segregate together, a genetic linkage between Fc receptor and the precipitable molecules will have been suggested.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5049(c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

F_1 Antiparental Cell-mediated Lympholysis in vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. M. Shearer, Ph.D.	Senior Investigator	IB, NCI
OTHER:	A.-M. Schmitt-Verhulst, Ph.D.	Expert	IB, NCI
	T. G. Rehn, M.D.	Clinical Associate	IB, NCI

COOPERATING UNITS (if any)

Gustavo Cudkowicz, M.D., Department of Pathology
State University of N.Y. at Buffalo

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

An in vitro system has been developed in which F_1 spleen cells generate cytotoxic activity directed against parental cells. The antigenic differences detected are controlled by the H-2D-Hh-1 region of the immune major histocompatibility complex. A number of positive correlations were demonstrated between hemopoietic graft rejection in vivo and F_1 antiparent cell-mediated cytotoxicity in vitro. These include genetic polymorphism, immunologic maturation, antigen-induced specific unresponsiveness, and selective but nonspecific abrogation of responsiveness either by antisera or antimacrophage agents. The observations that F_1 antiparent cytotoxic potential develops later than immunity to alloantigens and that selective abolition of F_1 antiparent cytotoxicity can be achieved without abrogation of reactivity to alloantigens suggests that these two cell-mediated immune phenomena are generated by different mechanisms. The F_1 antiparent cytotoxic system provides an in vitro model for elucidating the recognition and effector phases of hybrid resistance to parental hemopoietic grafts and may be useful in matching procedures for clinical bone marrow transplantation.

Project Description:

Objectives: Resistance of irradiated mice to proliferation of parental or allogeneic hemopoietic cells is regulated by multiple H-2 linked genes (Cudkowicz, in Genetic Control of Immune Responsiveness, McDevitt and Landy, Academic Press, 1972). These hemopoietic histocompatibility (Hh) genes are phenotypically expressed only in blood-forming and leukemic cells, are not codominantly inherited, and their products are capable of stimulating host-vs-graft reactions which are radioresistant in vivo. The objective of this study was to determine if cell-mediated cytotoxicity (CMC) could be generated in vitro in F_1 lymphocytes against parental cells; if so, to establish if the response involves reactivity against Hh determinants.

Methods Employed: Spleen cells from a number of F_1 mouse strains were sensitized in culture against parental spleen cells. The effector cells were assayed on ^{51}Cr-labelled tumor target cells expressing different H-2 haplotypes.

Major Findings: Similar to the in vivo F_1 hemopoietic graft rejection of parental cells, F_1 responding lymphocytes generated cytotoxic effector cells primarily against F_1 spleen cells of the $H\text{-}2^b$ parent and to some extent against determinants of the other parent. Genetic studies indicated that the recessive determinant in the parental strain maps in or near the D region of H-2, similar to that seen for the rejection of parental marrow by F_1 hosts. Other similarities between in vivo and marrow graft rejection and the in vitro generation of F_1 antiparental cytotoxic effector cells include: delayed maturation of F_1 antiparental immunity; specific abrogation of the response by pretreatment of F_1 mice with parental spleen cells; and nonspecific but selective abrogation of the response with either rabbit antimouse bone marrow serum or silica. The above criteria also distinguish F_1 antiparental cytotoxicity from allogeneic lympholysis. Differences between the F_1 antiparental reactions in vivo and in vitro include the observations that: (a) the in vitro cytotoxic effector cells but not in vivo graft rejection effector cells are Θ-positive; and (b) the immunological events in vitro but not in vivo appear to be dependent upon H-2 serologically detectable region products as well as the Hh determinant.

Significance for Biomedical Research and the Program of the Institute: Since the genes are expressed on both normal and neoplastic hemopoietic cells, this study may indicate some of the genetic and antigenic parameters involved in surveillance against leukemias. The adaption of an in vitro model for such studies permits analysis and dissection of these parameters not heretofore possible in vivo. The development of the in vitro system permits an investigation of possible Hh genes in humans.

Proposed Course of Project: Leukemic as well as normal marrow cells will be investigated as possible stimulator and target cells. Other established parameters of in vivo hybrid resistance will be compared with the in

vitro system. Effector cells generated in vitro will be used in vivo to determine if they have any protective effect against leukemias or could be relevant in immune surveillance against autologous tumors.

Publications:

Shearer, G.M. and Cudkowicz, G.: Induction of F_1 hybrid antiparent cytotoxic effector cells: An in vitro model for hemopoietic histoincompatibility. Science 190: 890-893, 1975.

Shearer, G.M., Waksal, H. and Cudkowicz, G.: An in vitro model for hybrid resistance to bone marrow grafts. In Good, R.A. and Dupont, B. (Eds.): Proceedings of "Symposium on the Immunobiology of Bone Marrow Transplantation," Transplantation Proceedings, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5050 (c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Structure and Function of the Constant Portions of Immunoglobulins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Segal, Ph.D.	Chemist	IB, NCI
OTHER:	E. Hurwitz, Ph.D.	Visiting Fellow	IB, NCI
	H. B. Dickler, M.D.	Senior Investigator	IB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.	2	0

SUMMARY OF WORK (200 words or less - underline keywords)

The interaction of immunoglobulins with the complement system has been studied. It has been observed that rabbit anti-DNP antibodies fix guinea pig and human complement to varying degrees, in the absence of antigen, depending upon the method of preparation. A technique for preparing non-fixing monomeric immunoglobulin has been developed, and in addition, monomeric anti-DNP has been prepared which fixes complement several hundred fold better than the "non-fixing" sample in the absence of antigen. Apparently the C_H2 domains of rabbit IgG can be perturbed, exposing the complement binding site and causing the molecule to trigger the complement cascade, independent of antigen.

Complexes of divalent affinity-labelled antibodies have been prepared and fractionated on the basis of size. The binding of these complexes to mouse spleen cells has been investigated. Monomeric IgG does not bind to more than about 5% of spleen cells, with the assay used. However small complexes containing 2-3 Ig molecules do bind to about 50% of the cells, even though these same complexes are not anticomplementary. Only very large complexes (>6 IgG) bind to a larger fraction of the cells. Covalently linked dimers have been isolated, and will be used to further characterize lymphocyte immunoglobulin receptors.

Project Description:

Objectives: 1) To investigate the interactions of the constant domains of immunoglobulins with the various effector systems employed in immune reactions. 2) To study the relationship of antigenic recognition to these interactions and to study the molecular basis of these phenomena.

Methods Employed: Organic synthesis, gel filtration, ion exchange chromatography, affinity labelling, affinity chromatography, polyacaylamide gel electrophoresis, complement fixation, fluorescent labelling of cells, equilibrium dialysis, radiolabelling of proteins.

Major Findings: 1) It has been observed that different preparations of monomeric immunoglobulin fix complement to differing degrees, in the absence of antigen, depending upon the method of preparation. We have developed techniques to prepare anti-DNP antibodies which will not fix complement appreciably but which become very anticomplementary upon addition of antigen (DNP-BSA), according to the traditional complement mechanism. However, monomeric anti-DNP can also be prepared by ammonia elution from a DNP-Sepharose column and this antibody preparation fixes complement almost as well as antigen-antibody complexes. A characteristic feature of normal antibody in the presence of antigen is that reduction of the interheavy chain disulfide bridges abolishes its ability to fix complement. Mild reduction also abolishes the fixing properties of the ammonia eluted material, other proteins (e.g. BSA or some other immunoglobulins) to become anti-complementary. When "turned-on" preparations are exposed to antigen, their complement fixing abilities increase only slightly, to about the same level of "normal" anti-CNP-antigen complexes. All evidence thus suggests that ammonia elution affects the same site on the immunoglobulin molecule that triggers the complement reaction.

The "turning-on" process does not appear to be the result of the formation of a monomer-dimer equilibrium. since fractionation of the "turned-on" material on Sephadex G-200 yields mainly a monomer peak, which is fully active, and which varies only slightly when separate fractions are assayed for fixing ability. The position at which the "turned-on" preparation elutes from a G-200 column is also the same as where normal anti-DNP elutes. Centrifugation of the "turned-on" material for 2 hr. at 100,000xg has no effect on its complement fixing activity.

The "turned-on" preparation precipitates with CNP-BSA about as well as the normal antibodies by agar double diffusion analysis, but the affinity for hapten as measured by equilibrium dialysis appears somewhat lower in the "turned-on" antibodies. Either the ammonia elution partially denatures the V-region sites, or perhaps results in the release of a lower affinity population from the DNP-Sepharose column.

It has previously been thought that IgG fixes complement by an aggregation mechanism, i.e. that by bringing several complement binding sites together in

close juxtaposition, Clq would bind firmly to the complex and trigger the complement cascade. Our results indicate that rabbit IgG has the potential of greatly increasing its ability to interact with complement, independent of aggregation, and suggest a mechanism involving an antigen-induced distortion of the C_H2 domains, leading to increased exposure of the relevant sites.

2) A series of divalent affinity labels for anti-DNP antibodies has been synthesized. One of these, $(BADL\text{-}Pro)_2$-EDA has been used to prepare a mixture of covalently and non-covalently linked anti-DNP complexes.

The mixture of complexes was fractionated on Sepharose 4B according to size, and the various fractions were tested for their ability to bind to mouse spleen cells. The cells were incubated with the complexes, washed, and treated with fluorescent goat anti-rabbit antibody, and the fraction of fluorescent cells determined. In general it was found that almost any size complex would appear to bind most strongly, but this assay also detects binding by fractions consisting mostly of dimers and trimers. Thus we can be reasonably certain that immune complexes containing as few as three immunoglobulin molecules will bind strongly to B cells.

Binding of complexes to a larger percentage of cells, i.e. to a subpopulation of T cells, was obtained only with much larger aggregates. These aggregates eluted between the void volume and IgM on the Sepharose column, and thus probably contain about 10 IgG molecules. In contrast to the antibody-divalent hapten complexes, neither monomeric anti-DNP, BADL-labelled anti-DNP, nor "turned-on" anti-DNP bound to greater than 5-10% of the spleen cells by the fluorescent antibody technique.

The complexes were also assayed for complement fixation. As has been previously reported, the smaller complexes--dimers and trimers--did not fix complement appreciably, whereas the larger fractions are very anticomplementary.

In order to measure the binding of immunoglobulins to cells more precisely, rabbit anti-DNP was labelled with about 1 mole of ^{125}I per mole of protein. When mouse spleen cells were incubated with the I-labelled antibody, washed, and assayed for bound radioactivity, it was observed that immunoglobulin would bind only when the divalent hapten was added, in agreement with the fluorescent labelling results. Attempts have also been made to detect monomer binding to spleen cells, by omitting the washing step. Preliminary studies using ^{125}I-labelled proteins other than immunoglobulins, showed that under our experimental conditions the cells could be removed from supernatant solutions, with about 2% of the supernatant solution remaining associated with the cells. We could never detect more than this amount of ^{125}I labelled antibody binding to the cells, which means that the affinity of mouse spleen cells for monomeric IgG is less than about 10^6.

Significance for Cancer Research and Program of the Institute: The recognition of foreign substances by immunoglobulins elicits a number of reactions which normally lead to their elimination from the body. Previous work by the principal investigator has contributed to knowledge of the molecular basis of antigenic recognition. This project proposes to investigate the ways in which recognition elicits the protective response of immunity. It is hoped that these studies will enhance our understanding of these processes and allow us to better control the immune reactions. Indeed, the observation that immunoglobulins can be artificially "turned-on" with respect to the complement system suggests that a more sophisticated approach toward controlling immunity (e.g. by passive immunization with "turned-on" antibody) may be possible in the future.

Proposed Course of Project: 1) The "turning-on" process of rabbit antibodies will be further investigated in order to elucidate the molecular basis of this effect. Other immunoglobulins of different classes and from different animals will also be tested for "turning-on". We also hope to determine at what stage the "turned-on" immunoglobulin operates in the complement cascade.

2) We ultimately hope to measure Fc receptor binding constants and determine the number of receptors per cell. In order to do this, pure covalently linked dimer will be isolated, labelled, and its binding to cells measured. Hopefully the Fc receptors on various cell types can then be characterized by measuring the inhibition of dimer binding by immunoglobulins of differing class and origin.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5051(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cell-mediated Immunity to Spontaneously Arising Syngeneic Murine Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. M. Shearer	Senior Investigator	IB DCBD
OTHER:	R. B. Levy	Biologist	IB DCBD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

An AKR/J murine carcinoma-like tumor was generated in tissue culture from an undifferentiated fibrosarcoma. An autologous immune response was generated against the carcinoma in vivo and in vitro. Subcutaneous injection of low numbers of the carcinoma-like cells resulted in a period of tumor growth, followed by a period of tumor regression. Depression of a number of T-cell functions were observed during the period of tumor growth, whereas T-cell functions returned to normal levels during the regression phase. Cell separation procedures revealed that a population with suppressor cell characteristics directed at T-lymphocyte functions could be identified during the period of tumor growth.

PHS-6040
(12-75)

Project Description:

Objectives: The major objectives of this proposal are twofold: 1) to determine whether H-2 cell surface proteins controlled by genes mapping within the murine major histocompatibility complex (MHC) are a necessary component in the generation of autologous effector cells against virally infected targets; and 2) to investigate the immunological regulatory events subsequent to this recognition, which result in the generation of suppressor and/or effector cell populations.

Methods Employed: A three-step, preparatory and analytical procedure is being used in analyzing and contrasting the significance of suppressor cells in two spontaneously arising murine neoplasms of AKR origin: one, a viral-producing, in vivo-arising, undifferentiated fibrosarcoma, and the second, a nonviral-producing, in vitro-arising, undifferentiated carcinoma-like cell type.

1. Generation of the suppressor cell: Tumor cells are injected both intraperitoneally or subcutaneously as a means of generating suppressor cells. Attempts will also be made to generate these cells in vitro by varying the duration of the sensitization period.

2. Isolation and purification of the cell: The fractionation of cell suspensions into enriched T-cell, B-cell, adherent cell, etc., and other lymphocyte populations are obtained by passage over nylon wool or glass bead columns. Subpopulations will be further subdivided by 1G velocity sedimentation or separation on the fluorescent activated cell sorter.

3. Analyses of the suppressor cell: The supressor cell population is characterized by its ability to inhibit the following: (a) the immunization of normal spleen cells to the tumor cell (afferent limb inhibition); (b) the mitogenic response of normal spleen cells (central limb inhibition); (c) the cytotoxic response of immunized spleen cells against the tumor cell (efferent limb inhibition); (d) the antibody producing capability of normal spleen cells; and (e) the normal rejection of sublethal tumor doses in vivo.

Major Findings: Following 3-4 in vitro passages of a spontaneously arising, undifferentiated fibrosarcoma (AKR/J), a second cell type repeatedly predominated the cultures. In contrast to the fibrosarcoma, this second cell (carcinoma-like in a number of its chromosomal and growth characteristics) generated an effective immune response in the syngeneic host. Type C virus was observed in micrographs of the fibrosarcoma, but not carcinoma-like neoplasm. Positive reverse transcritase findings in low passaged (fibrosarcoma) but not high passaged (carcinoma) cultures substantiated the morphological findings. Subcutaneous injection of low numbers of carcinoma-like cells resulted in a period of increasing (progressor) tumor size, followed by spontaneous regression (regressor). During the progressor period, a number of T-lymphocyte responses in the spleens of these animals become markedly depressed (PHA, Con A stimulation; mixed lymphocyte reactivity; T-dependent

antibody response). Similarly, spleen cells from these animals did not manifest cytotoxicity against the tumor cell in vitro. In contrast, each response gradually approached normal levels as the tumor regressed, including the ability to lyse the tumor cell in vitro. The removal of a population of adherent cells from spleens during the progressor period restored PHA and Con A responsiveness to normal levels. Additionally, the mixture of progressor spleen cells with normal spleen cells inhibited their mitogen responsiveness, mixed lymphocyte reactivity, and ability to generate cytotoxic effector cells against alloantigens.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the mechanisms influencing immune reactivity against spontaneously arising in vitro and in vivo neoplasms are of importance in cancer research. The contribution of virus to the immunogenicity of the transformed cells will be investigated to determine whether such activity is directed against virus alone, or against viral-modified, major histocompatibility-linked autologous cell surface components. The significance of viral expression in activating suppression and/or effector mechanisms relevant for the immunity observed in the tumor-bearing host may be of relevance in tumor immunity. The contribution of genes regulating immune response potential to such antigens to determine the role of immune surveillance and host susceptibility in autologous arising neoplasms are also probably important. In the long term, this program is part of the overall aim of the principal investigator's laboratory, which is to examine autologous immune reactions as a means of understanding the problems associated with immune surveillance against spontaneous neoplasia.

Proposed Course of Project: The observed patterns of cytotoxicity will determine whether the viral moieties plus H-2-controlled autologous cell surface components are recognized as the immunogenic unit in this response. If viral infection is found to be necessary (i.e., virally modified syngeneic fibroblasts generate autologous effector cells against identical targets) but not sufficient in itself (i.e., virally infected syngeneic fibroblasts do not induce autologous effectors against virally infected non-H-2 matched targets) to obtain cytotoxicity, H-2 homology between stimulating and target cells would indicate that viral modification of an H-2 gene product on the cell surface is the necessary antigenic unit for generating autologous recognition against the neoplastic cell. The second part of the project will be directed at investigating the regulatory events subsequent to the recognition of tumor by the autologous host.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5052 (c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Psychosocial Factors in the Prognosis of Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. N. Rogentine, M.D., Senior Investigator, Immunology Branch, NCI

OTHER: J. Docherty, M.D., Clinical Associate, Adult Psychiatry Branch, NIMH

D. van Kammen, M.D., Chief, Unit on Neuropsychopharmacology, Adult Psychiatry Branch, NIMH

COOPERATING UNITS (if any)

Dr. Wm. Bunney, NIMH, Dr. Bernard Fox, NCI, Dr. Wm. Terry, NCI, Dr. Steven Rosenberg, NCI, Dr. Richard Fisher, NCI

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6 IB-0.5, APB-0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effect of various psychosocial factors on host resistance to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from those who either remain disease-free or have a delayed recurrence.

Project Description:

Objectives: Malignant melanoma is an unpredictable disease. Its course can range from rapid demise to spontaneous remission. The reasons for this variability are not known.

Psychosocial factors are known to play a role in the development of a wide variety of medical illness: e.g., duodenal ulcer and coronary heart disease and there is a growing body of evidence that such factors predispose normal individuals to malignancy, or modify its course once established. It is thus conceivable that psychosocial factors may account for some of the variability in the clinical course of malignant melanoma.

Clinical Stage II melanoma is defined as a local primary malignancy with extension to regional nodes. Surgical resection can render these patients disease-free. Many of such "cured" patients subsequently develop Clinical Stage III disease (distant metastases), usually within two years and go on to die. But for a period of time there exists a unique group of patients with known cancer, potentially cured, but at risk of recurrence. Since much previous psychosomatic work has been retrospective in type and fraught with the inherent methodological flaws of that approach, this patient group presents a uniquely important opportunity to carry out a well controlled prospective study on the influence of psychosocial factors on disease progression.

Our purpose is to develop a detailed characterization of and to test several hypotheses regarding the psychosocial factors which distinquish those patients who suffer recurrence of the malignancy from those who either remain disease-free or who have a delayed recurrence.

Methods Employed: We plan to employ the following methods in generating a psychosocial profile of these patients.

I. Psychiatric Interview

Each patient will be seen following surgical recuperation or during clinical staging for an interview of approximately an hour and a half duration. The purpose of this interview will be to gather data regarding the patient's past childhood, occupational and marital history, to identify the relevant psychosocial factors possibly involved in precipitating the current illness, and to make an assessment of the patient's attitude toward the future, his involvement, integrity of ego functions and object relations.

II. Standardized Questionnaire

1. MMPI - This is an empirically derived and extensively studied instrument which generates a profile of the patient in several dimensions of personality. This test has very high reliability and will be administered once.

2. Holmes and Rahe Schedule of Recent Experience - This is a 52-question form used to assess the magnitude of life change the patient has experienced during a given time interval. It has had the benefit of extensive validation study and previous research has demonstrated a high correlation between the magnitude of life change measured by this instrument and the onset of subsequent illness. This scale will be administered initially and at 2 month intervals.

3. SCL-90 Symptom Checklist - This is a self-administered form which checks for the presence of a wide variety of psychological and neuro-vegetative symptoms. It will be administered initially and at 2 month intervals.

4. Rotter Internal - External Locus of Control Scale - This is a 29-item self-administered form which is currently being widely researched. It appears to be a personality dimension with correlates from the neurological to social level of organization. This test also has very high reliability and will be administered only once.

III. Psychobiological Measures

The concurrent intensive study of the immune system being conducted with these patients will also allow us to gather data which may suggest a mediating mechanism for the effect, if any, of psychosocial factors on the progression of the disease. It is possible that psychosocial stress may modify immune responses, for example, via at least two stress responsive hormones, cortisol and the catecholamines. Both these hormones modify immune responses. Since the immune system seems to play a role in the development and containment of cancer, psychosocial factors may influence malignancy via the intermediary of the immune system.

In addition to the assays of immune competence we will collect on the initial admission 24-hr urine samples for 17 hydroxycorticosteroid and 17 ketosteroid levels, and VMA excretion.

Once these tests are conducted and scored, the results will be correlated with the patient's stage of disease progression. Our aim is to construct a psychosocial and psychobiological profile of "progressors" and "non-progressors".

Major Findings: To date we have entered 25 patients into the study. Five of these patients have relapsed, 20 remain disease free. The relapsed group is too small to draw meaningful comparisons with psychosocial variables. We will wait until a larger number of patients have relapsed before analyzing our data.

Significance for Biomedical Research and the Program of the Institute: An understanding of all environmental and host factors in oncogenesis is necessary to progress in cancer research. Psychosocial factors can influence

disease occurence and prognosis. The magnitude of the impact of these factors on host resistance to neoplasms has never been adequately documented, in large part due to the retrospective design of previous clinical experiments. This study avoids that problem: it is a prospective study. Thus we shall be able to make stronger statements about possible psychosocial correlates with cancer upon its completion. Identification of psychological or social stress factors in the promotion of tumor growth is clearly of importance basically as well as for its therapeutic implication. Just as immunotherapy is designed to strengthen host resistance to tumor, psychotherapy could conceivably be used for the same reason.

Proposed Course of Project: We propose to enter about 100 patients into the study. We will look at data from the first 50 patients for possible correlations when enough of them relapse. Any correlations found will be the basis of hypotheses which the second group of 50 patients will test.

If clear cut correlations between certain psychosocial variables and disease progressions are found, we will consider studying appropriate psychotherapeutic measures in the treatment of melanoma.

In addition any correlations between psychosocial factors and endocrine or immune variables will be sought and appropriate further clinical experiments designed to investigate their nature more fully.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5053(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Resistance to AKR Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. Zatz, Ph.D.	IPA Guest Worker	IB, NCI
OTHER:	A.-M. Schmitt-Verhulst, Ph.D.	Expert	IB, NCI
	G. M. Shearer, Ph.D.	Senior Investigator	IB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.95	.95	

SUMMARY OF WORK (200 words or less - underline keywords)

The genetic factors underlying resistance to growth of AKR lymphoma cells have been investigated. An F_1 hybrid (AKD_2F_1) of AKR/J mice, as well as H-2 compatible (AKR/Cu, CBA) mice show prolonged or indefinite survival following injection of AKR/J lymphoma cells. This in vivo resistance correlates with in vitro assays of cell-mediated cytotoxicity (CMC). Resistance of $AKDF_1$ mice is dose dependent, and not absolute; eventually, these mice succumb to leukemia. The AKR/Cu mice, which are known to differ from the AKR/J lymphoma donors only at the 9th chromosome, show more profound resistance at doses of less than 10^6 AKR/J lymphoma cells. The AKR/Cu mutant does not have any detectable tumor growth. CBA/J mice, which are also H-2 compatible, show complete resistance to even greater doses of AKR/J lymphoma.

The F_1 in vitro CMC can be elicited as a weak primary response, detectable at effector:target ratios of ca:80/1 on tumor targets. In contrast, the AKR/Cu α AKR/J or CBAαAKR/J CMC is detectable only after in vivo priming. However, the secondary response in vitro is almost as strong as a primary allogeneic response. The only known antigenic difference between AKR/Cu and AKR/J mice is the Thy antigen. Both strains are high Gross virus producers and have a high spontaneous incidence of T-cell lymphoma. The basis of this strong resistance to the reciprocal strains tumor is of some interest.

Project Description:

Objectives: The objectives of this project are to define the basis for resistance or susceptibility to growth of AKR lymphoma cells in AKR/J mice, F_1 hybrids of AKR/J, and H-2K compatible mice.

Methods Employed:

1. In vivo resistance. The growth of AKR/J lymphoma cells in AKR/J, AKD_2F_1/J, AKR/Cu and CBA/J mice were studied. Doses of 10^3-10^6 lymphoma cells from spleen or thymus of AKR/J mice with spontaneous lymphocytic leukemia were injected iv.v into the recipients. Survival was compared between groups.

2. In vitro resistance. Spleens of normal young mice, or of mice previously challenged with AKR/J lymphoma cells, were assayed in vitro for cell mediated cytotoxicity (CMC). Cells were cultured in vitro for 5 days with appropriate ratios of 2000R irradiated cell immunogens. After 5 days the cells were harvested and assayed for their ability to kill ^{51}Cr labelled tumor targets (RDM4, derived from AKR/J) or appropriate PHA-stimulated spleen lymphocytes.

Major Findings:

1. AKD_2F_1 resistance: $AKRxDBA_2$ (H_2K, Thy 1.1 x H2-d, Thy 1.2) F_1 hybrids show selective resistance to growth of AKR/J (H-2K/1.1 Thy) lymphoma cells at doses of 10^3-10^5. Survival is prolonged anywhere from 1 week to >1 month, depending on the experiment and dose employed; eventually AKD_2F_1 mice develop lymphoma and die.

2. AK/Cu resistance. AKR/Cu mice, derived from the same common stock as AKR/J have a mutation of chromosome 9, rendering them Thy 1.2 instead of Thy 1.1. Other changes are associated with this mutation, i.e., a difference in an esterase enzyme. These AKR/Cu mice, which resemble the AKR/J mice in their high titer of Gross virus, and spontaneous incidence of lymphomas are resistant to growth of AKR/J tumor cells. 100% of mice injected with 10^5 cells survive indefinitely. At doses of 10^6 cells, 80% of mice do not get lymphoma. The reciprocal experiment gives similar results, i.e., AKR/Cu lymphoma cells injected into AKR/J mice also do not cause death of recipients.

3. CBA resistance. CBA mice which are H-2K, but Thy 1.2, also differ from AKR/J mice at multiple minor histocompatibility loci. These mice are even more resistant than are AKR/Cu mice to growth of AKR/J lymphoma. 100% of mice survive injection of $5x10^6$ AKR/J lymphoma cells.

The results thus far indicate that the F_1 resistance may be due to an Hh antigen associated with the K end of H-2K. It is necessary for the targets to express H-2K antigens in order to detect 1° in vitro sensitization of F_1 cells to AKR normal or leukemic cells.

The basis of the AKR/Cu resistance to AKR/J and vice versa may, or may not be due to the Thy antigenic difference. AKR/Cu mice primed in vivo to AKR/J cells will develop secondary CMC in vitro against some, but not all, H-2K immunogens. Likewise, some but not all H-2K targets will be lysed by AKR/Cu cells, primed in vivo and in vitro to AKR/J. These discrepancies between K-2K cells cannot be solely attributable to the Thy antigenic type. Therefore, it may be that H-2K Thy and additional antigen(s), coded for by the 9th chromosome, are responsible for the strong in vivo resistance to growth of AKR/J lymphoma.

CBA. The model of CBA resistance to AKR/J lymphoma appears similar to the AKR/Cu system, although CMC is stronger, attributable to the greater degree of antigenic disparity.

Significance for Cancer Research and Program of the Institute: The AKR/J mouse provides a model for study of acute lymphocytic leukemias of T-cell origin; similar T-cell leukemias, presenting with thymoma, have been described in man. The question of leukemogenesis in AKR/J mice is complex. Several factors play critical roles in determining the onset and incidence of murine leukemia. These factors are 1) the animals titer of Gross leukemia virus; 2) the H-2 type; 3) the thymus epithelium. It is hoped that by comparing the resistance of the AKR/J mouse, and of strains which are genetically related, that an understanding of the genetic base of resistance to lymphoma may be achieved. These studies also offer the opportunity to pinpoint antigenic differences, expressed on lymphoma cells, which can elicit effective host immunity.

Proposed Course of Project:

1. F_1 resistance. The major emphasis will be on following changes in the level of CMC following in vivo challenge with lymphoma cells. Since the mice initially resist growth of cells, but eventually succumb, something may be learned of the changes in level of immunity paralleling the in vivo events.

2. AKR/Cu and CBA/J resistance. The in vitro studies will be extended in order to define the antigenic differences between the AKR/J, AKR/Cu and CBA cells, which are responsible for eliciting in vivo resistance.

Hybrids of AKR/JxAKR/Cu and AKR/JxCBA/J are being prepared in order to determine whether the F_1 state will confer resistance to the high spontaneous incidence of leukemia observed in AKR/J and AKR/Cu mice.

3. AKR/Cu and AKR/J mice will be challenged with the reciprocal lymphoma cells and observed for long term survival. It will be of considerable interest to determine whether by priming against the reciprocal strain, one can prevent or reduce the strains' own high spontaneous incidence of lymphoma.

Publications.

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-5054(c)01-I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mouse macrophage activation with BCG: relationship to antitumor activity in vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. F. Nathan, M.D.	Clinical Associate	IB, NCI
OTHER:	W. D. Terry, M.D.	Associate Director	IB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Adherent peritoneal cells from normal mice and mice infected with BCG were studied in vitro for their interaction with 15 different mouse tumor lines. The results were compared with assays for other features of macrophage activation, notably phagocytosis. A previously unsuspected degree of heterogeneity was revealed, both in terms of the patterns of "macrophage"-tumor interaction, and in terms of cell types present among adherent peritoneal cell populations and previously referred to simply as "macrophages." Specifically, four patterns of adherent peritoneal cell-tumor interaction were seen, ranging from inhibition of tumor proliferation to profound stimulation. The stimulatory effect of normal adherent peritoneal cells was much greater than that of such cells from BCG-treated mice, and was closely paralleled by the effect of 2-mercaptoethanol. Adherent peritoneal cells from BCG-treated mice displayed defective phagocytosis, due in part to the presence of increased numbers of nonphagocytic adherent cells. The latter were isolated and shown to have potent anti-tumor activity, which might have accounted for all that displayed by the whole adherent cell population. These cells may represent a heretofore undescribed subpopulation with certain properties of both macrophages and B lymphocytes.

Project Description:

Objectives: Previous studies showed that treatment with a variety of agents, thought to activate macrophages, promoted resistance to tumors in vivo, in correlation with increased anti-tumor activity of "macrophage" populations taken from such animals and studied in vitro. Other studies have demonstrated a wide variety of functional, morphological, metabolic and surface alteractions of macrophages after "activation" by different means. There is little information, however, to indicate which, if any, of the known features of "activation" may be relevant to the anti-tumor activity of activated macrophages, nor even which cells among the population called "macrophages" may be most important for anti-tumor activity. Understanding of these relationships has been hampered by a lack of sufficiently quantitative assays for various macrophage functions. The objectives of the study were to develop in vitro methods for the assay of anti-tumor activity and for certain other macrophage functions, such as phagocytosis, and then to identify relationships between activation and anti-tumor activity which might point to the existence of subsets of cells among "macrophage" populations.

Methods Employed: Mice were injected with BCG, their peritoneal cells collected, and the adherent or nonadherent cells allowed to interact with a variety of mouse lymphoma or fibrosarcoma tumors in microcultures. Inhibition of tumor cell proliferation was assessed by uptake of tritiated thymidine. Assays for the phagocytosis of a variety of particles were either modified or newly developed to allow quantitation under optimal dose-time conditions, rarely attained in previous studies. These particles included radiolabelled starch granules and bacteria, graphite dust and latex beads. Peritoneal cell populations were also studied for a variety of histochemical and surface markers, such as surface immunoglobulin, complement receptors and Fc receptors. Mice treated with a variety of other agents were also studied. Glucose metabolism and the roles of hydrogen proxide and 2-mercaptoethanol in macrophage-lymphoma cell interaction were examined. Finally, a means was devised for the physical separation of certain subsets of adherent peritoneal cells.

Major Findings: Four different patterns of macrophage-tumor cell interaction in vitro were identified, using approximately 15 different tumors. These ranged from tumor inhibition, the expected pattern, to profound stimulation of tumor cell proliferation, a previously unsuspected pattern consistently seen in this study using a certain group of lymphomas. A third group displayed no detectable effect of interaction with macrophages, and a fourth group was intermediate between the last two. It was discovered that the proliferative effect of 2-mercaptoethanol on certain of these tumors closely paralleled the proliferative effect of normal macrophages. Macrophages from BCG-infected mice were far less stimulatory than those from normal mice when allowed to interact with tumors of the second and fourth groups above.

Using a variety of particles, a phagocytic defect was identified in adherent peritoneal cells from BCG-treated mice. This appeared to have two components: an increased number of nonphagocytic adherent cells among the peritoneal cells of such mice, and a decrease in the uptake of particles by the remainder of the adherent cells. The nonphagocytic adherent cells were isolated and shown to have marked anti-tumor activity which could not be attributed to the isolation technique. The activity of this subpopulation was such as possibly to account for all the anti-tumor activity of the total adherent cell population. Marker studies suggested that the nonphagocytic adherent cells represent a newly identified subpopulation with some properties of macrophages and others of B lymphocytes. The possibility is being pursued that the more typical macrophages were responsible for the proliferative effects seen with many of the tumors studied.

Significance for Biomedical Research and the Program of the Institute: These studies suggest that the pattern of interaction of macrophages with tumor cells is more heterogeneous than previously suspected. In this system, BCG treatment results in increased tumor inhibitory activity, decreased stimulatory activity, or no change, depending on the tumor studied (and other variables). An unexpected heterogeneity among adherent peritoneal cells was also revealed. Strong support was given to the hypothesis that anti-tumor activity of "macrophage" populations used here may in fact reside with a small but potent subpopulation, which may not even be macrophages in the usual sense. This would place in a different light the question of the relationship of various tests of macrophage function (such as phagocytosis) to the anti-tumor activity usually attributed to macrophages. Thus, understanding may be fostered in two directions: concerning the basic biology of host defense against tumors, and concerning the tests with which to monitor the effect of immunotherapeutic agents, such as BCG, on host lymphoid-mononuclear cell function.

Proposed Course of Project: Further characterization of peritoneal cell subpopulations is under way. If time permits, the presence of similar subpopulations will be sought in other organs, such as spleen, and models will be sought with which to assess the functional importance of these cell types in vivo. Study of the role of hydrogen peroxide metabolism in macrophage-tumor interaction will be continued. The possibility that the nonphagocytic adherent peritoneal cell may have suppressor activity in in vitro sensitization for T lymphocyte mediated cytotoxicity will be studied in collaboration with Dr. Richard Hodes. Finally, the mechanism by which BCG can induce a phagocytic defect is under study.

Publications:

Nathan, C. F. and Terry, W. D.: Differential stimulation of murine lymphoma growth in vitro by normal and BCG-activated macrophages. J. Exp. Med. 142: 887-902, 1975.

Nathan, C. F., Hill, V. M. and Terry, W. D.: Isolation of a subpopulation of adherent peritoneal cells with anti-tumor activity. Nature, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER NCI Z01 CBD 5055 (c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Regulatory Influences on Cell-Mediated Immune Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Hodes, M.D.	Head, Immunotherapy Section	IB NCI
OTHER:	L. M. Nadler, M.D.	Clinical Associate	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.75	.75

SUMMARY OF WORK (200 words or less - underline keywords)

It has been demonstrated that the in vitro culturing of normal murine spleen cells results in the generation of active suppressor cell activity. This activity is assayed as the ability to suppress the response of otherwise competent cells to alloantigens in cell-mediated lympholysis. A T cell subpopulation has been shown to be essential to this suppressor activity. Furthermore, the suppression has been shown to effect responses to transplantation antigens including alloantigens, "modified self" determinants, and syngeneic tumors.

Project Description:

Objective: In addition to the cells directly responding to specific antigenic stimuli, there has been increasing data presented to suggest that additional cellular influences may be exerted at a regulatory level. It has been the objective of current studies to examine the role of such regulatory influences in the response to transplantation antigens of several classes.

Methods Employed: The methodology employed has been previously described in detail. Culture techniques were used to generate cell-mediated cytotoxicity in vitro to alloantigens, to "modified self" antigens, or to syngeneic tumor cells. The effect of other cell populations upon this response was then investigated. Newer techniques were developed to allow the study of possible soluble factors. Diffusion chambers, including Marbrook chambers and Karush chambers, were employed with membrane separation of various cell populations in order to analyze the need for cell contact or proximity in the responses being studied.

Major Findings: It was observed that culturing normal murine spleen cells in vitro, in the absence of intentional antigenic stimulus, resulted in the loss of their ability to subsequently respond to allogeneic cells by the generation of cell-mediated cytotoxicity. In addition, these "precultured" cells were capable of actively suppressing the response of otherwise responsive cells. This suppression has been shown to depend upon a small nylon-adherent subpopulation of splenic T cells. It is active at the level of sensitization. Extensive efforts have failed to associate this suppressive activity with any soluble factor in the supernatant, or diffusing across various membranes; extracts from actively suppressive cells have also been inactive. Suppression has been observed in the responses to alloantigens and to syngeneic tumor cells as well.

Significance to Biomedical Research and the Program of the Institute: States of in vivo tolerance or hyporesponsiveness have been associated with active suppressive populations in a number of systems. In addition, the possible role of specific or nonspecific immune suppression in the evolution or progression of malignancies has been raised. The studies described above may give some information concerning the mechanism of such in vivo immunosuppression and possible means of modifying the suppression as a therapeutic possibility.

Proposed Course of Project: Several aspects of the suppression which has been observed are now under investigation. These include questions of the effect of sensitization to transplantation antigens upon subsequent generation of suppression in vitro; questions of the genetics of the suppression; and investigations of the effect of in vitro cultured cells upon in vivo functions such as graft-versus-host responses, allograft responses, and tumor growth.

Publications:

Hodes, R.J. and Hathcock, K.S.: In vitro generation of suppressor cell activity: Suppression of in vitro induction of cell-mediated cytotoxicity. J. Immunol. 116: 167-177, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5056(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Specific Removal of Histocompatibility Antigen-Reactive Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Neefe, Jr., M.D.	Investigator	IB NCI
OTHER:	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

Since receptors for transplantation antigens may be visualized directly on unsensitized cells with the use of anti-idiotypic antisera, it should be possible to bind these cells to a cellular immunoabsorbent bearing the appropriate antigens. The cellular immunoabsorbents were monolayers of normal spleen cells attached to polystyrene coated with poly-L lysine. An in vitro murine model of cellular immunity involving generation of cytotoxic cells in mixed lymphocyte culture and assay using ^{51}Cr-labelled spleen cells as targets was used. Appropriate monolayers reduced cytotoxic activity of sensitized populations by a median of 94% but were unable to reduce the capacity for sensitization of normal cells or cells past the peak of primary immunization. On the contrary, absorption of these populations usually resulted in nonspecific augmentation up to 100 fold in the ultimate cytotoxic activity.

Project Description:

Objectives: Several lines of evidence indicate clonal diversity of T lymphocytes reactive to transplantation antigens and recently it has been possible to visualize directly receptor-bearing lymphocytes in unsensitized populations. If precursors of cells reactive to transplantation antigens bear receptors to those antigens, it should be possible to eliminate them from a lymphoid population by adherence to a solid phase immunoadsorbent bearing those antigens. Some previous studies of this principle have supported its validity, although others have not.

In addition to the obvious usefulness of such a technique and principle for study of the generation of cellular immunity, some clinical applications are of importance, as in specific tumor immunotherapy. Cells sensitized by whatever means against tumor-associated antigens could prevent the appearance or recurrence of tumor, but they must also be rendered incapable of causing graft-vs-host disease by elimination of any capacity to react to the transplantation antigens of the host.

Methods Employed: Studies were performed using normal mouse spleen cells. Reactivity to transplantation antigens was assessed at the effector stage by the capacity to lyse ^{51}Cr-labelled targets in a 4 hr assay and at the sensitization stage by the capacity to generate specifically cytotoxic cells in mixed lymphocyte culture. Cellular immunoadsorbents were monolayers of normal spleen cells attached to polystyrene coated with poly-L-lysine.

Major Findings: Spleen cell monolayers remove cytotoxic cells from a previously sensitized population efficiently and specifically. Eighty-eight to greater than 98% reduction of cytotoxic activity by a single absorption was achieved with a median in 14 experiments of 94%. Only a monolayer carrying the histocompatibility antigens to which the cytotoxic cells are sensitized is effective in absorption; cytotoxic cells reactive to the K end of the murine major histocompatibility complex may be separated from those reactive to the D end by absorption on appropriate recombinant strain monolayers. Apparent absorption is not caused by release of monolayer cells to serve as competitive inhibitors in the cytotoxic assay; direct enumeration of monolayer cell contamination of the absorbed cytotoxic population with the use of H-2 typing sera and a fluoresceinated antiglobulin specific for these typing reagents revealed insufficient contamination to block cytotoxicity as assessed separately by the addition of graded numbers of cold targets to the cytotoxic assay.

In contrast to the findings with cytotoxic cells, unsensitized cells could not be absorbed, and in fact, exposure to a monolayer of any antigenic constitution nonspecifically increased the ultimate activity of the population after specific sensitization. Since *in vitro* sensitization drives cells toward the more absorbable cytotoxic state, it was thought that precursors might also become absorbable after sensitization of the population. Therefore, cytotoxic cells after the peak of activity were absorbed and residual cytotoxic activity was removed. These cells were then boosted *in vitro* and

tested again for cytotoxic activity. Again, rather than a decrease in activity, an augmentation up to 100 fold resulted from absorption. The augmentation was nonspecific, since it was seen after absorption on effector-type or target-type monolayers or on uncoated plastic. The augmentation presumably reflects another phenomenon, possibly the adherence of nonspecific suppressors, but it illustrates that removal of precursor activity was insufficient to prevent regeneration of cytotoxic cells.

Significance to Biomedical Research and the Program of the Institute: Transplantation may be useful for cancer therapy in several settings. Transplants of lymphoid cells appropriately sensitized may control the appearance or growth of tumors. Transplants of organs may replace organs ravaged by tumors. Rescue with transplants of bone marrow may permit use of doses of anticancer chemotherapy which would otherwise be lethal. In each setting, success depends in part on specific control of responses to histocompatibility antigens. This project examines one means for eliminating these responses specifically.

Proposed Course of Project: Since receptors are demonstrable on precursors of transplantation antigen-reactive cells, continued efforts will be made to find conditions under which the cells bearing them may be effectively bound to adsorbents. In particular, the possibility that all precursors of cytotoxic cells generated in secondary sensitization must pass through a cytotoxic stage after primary sensitization suggests that absorption at the height of primary response may be more effective in rendering the population specifically nonresponsive. Alternatively, the possibility that absorption of a precursor in the presence of a poorly absorbed amplifier cell may be nullified by a synergistic amplifier-precursor interaction may be examined by conducting absorptions of populations in which the precursor is not present in excess. Finally, the suppressive effects observed must be understood and controlled before statements about the absorbability of precursors may be made with absolute certainty.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5057 (c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Genetics of Proliferation and Cytotoxicity Generated in Mixed Lymphocyte Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Hodes, M.D.	Head, Immunotherapy Section	IB NCI
OTHER:	G. M. Shearer, Ph.D.	Senior Investigator	IB NCI
	A.-M. Schmitt-Verhulst, Ph.D.	Expert	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

SUMMARY OF WORK (200 words or less - underline keywords)

Mixed lymphocyte cultures (MLC) have been studied in which the responding and stimulating strains differed at discrete genetic regions. Differences limited to the D region of the major histocompatibility complex were sufficient to produce both proliferation and the generation of cell-mediated cytotoxicity in vitro. Included in these experiments were combinations differing only at the site of a point mutation in the D region; this difference was also adequate to generate strong responses.

Project Description:

Objective: This work was intended to define the precise genetic differences between responding and stimulating cells in mixed lymphocyte cultures (MLC) which are adequate to generate proliferation or specific cell-mediated cytotoxicity.

Methods Employed: MLC's were carried out in microculture system to analyze proliferative responses by incorporation of tritiated thymidine, and in macroculture for the generation of cytotoxic effector cells. Cytotoxicity was assayed by chromium release assays of target cell destruction. Recombinant and mutant mice were used in combinations such that the genetic differences between strains was always limited to either the entire major histocompatibility complex (MHC) or to discrete subregions at the D end of the complex.

Major Findings:

1) Genetic differences between responding and stimulating strains limited to the D end of the MHC (with no I region differences) were sufficient to produce significant proliferation and cell-mediated cytotoxicity.

2) The efficiency in stimulating proliferation or cytotoxicity across isolated D region differences was generally less than that in stimulating across broader MHC differences. When the mutant da allele was involved, however, stimulation across a limited D region difference was as efficient as broad MHC stimulation.

Significance to Biomedical Research and the Program of the Institute: The demonstration that discrete genetic differences, including one limited to a point mutation, may be adequate to stimulate both proliferation and the generation of cell-mediated cytotoxicity may be relevant to the situation involving host and autochthonous tumor. Here, the ability of tumor-specific antigenic differences to activate responding host cells may determine the ability of that host to generate an effective immune response directed at the tumor. Specifically, the fact that the genetic differences studied were limited to the D end, and involved no known I region differences, confirms the fact that genetic regions other than the I region and the non-MHC M locus may be adequate activating loci in these cell-mediated response systems.

Proposed Course of Project: The studies described above have concluded the immediate goals of this specific project.

Publications:

Hodes, R.J., Schmitt-Verhulst, A.-M., Hathcock, K.S. and Shearer, G.M.: Mixed lymphocyte reactivity and cell-mediated lympholysis to D-end differences of the murine major histocompatibility complex. Scand. J. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5058 (c)-02 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunoregulation of Antibody

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. O. Cowing, Ph.D.	IPA Investigator	IB NCI
OTHER:	H. B. Dickler, M.D.	Senior Investigator	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.125	1.125	0

SUMMARY OF WORK (200 words or less - underline keywords)

The Ig subclass specificity of immunoregulation by Ab will be examined with the use of subclass purified Ab to an Ag under Ir gene control. The fine specificity of Fc receptors on immunocompetent cells will be determined in an effort to assess the role of these receptors in activation of the cells by antigen and control of the immune response.

PHS-6040
(12-75)

Project Description:

Objectives: (1) To examine immunoregulation by antibody purified into class and subclass of Ig; (2) To determine the subclass specificity of Fc receptors on murine immunocompetent cells and; (3) To determine the role, if any, of Fc receptors in the induction or suppression of the immune response.

Methods Employed: (1) Immunization of responder and non-responder mouse strains with (T,G) A...L by various protocols. (2) Purification of Ab into class and subclass by iso-electric focusing and solid phase immunoadsorption using heavy chain specific antisera. (3) Use of Ag-Ab complexes and indirect immunofluorescence on purified cell populations. (4) In vitro and in vivo assays for the response of the cells to antigen: proliferation, Ab production, CMI, helper function, suppressor activity.

Major Findings: None. The project has just been started.

Significance to Biomedical Research and the Program of the Institute: Characterization of the conditions under which Ab stimulates or suppresses either CMI or Ab production would increase the potential for control of the immune response. Determination of the fine specificity and function of Fc receptors on immunocompetent cells would clarify the mechanisms of antibody dependent cell-mediated cytotoxicity and of Ab-dependent tumor cell enhancement, i.e. immune mechanisms which may play a role in the control or lack thereof of malignant cells.

Proposed Course of Project: If antisera can be raised and purified into class and subclass specific antibody, these reagents will be used to determine the regulatory role of the Fc portion of the molecule in stimulation and suppression of immunity. The use of a defined Ag under Ir gene control should allow determination of the effects of Ab on T cells and B cells in vivo, and cell surface markers and growth properties will be used to assess the effects of Ab on the functions of T and B cells, null cells and macrophages in vitro. The subclass purified reagents will be used in Ag-Ab complexes to determine the fine specificity of Fc receptors on the above cells and to relate the presence of Fc receptors on immunocompetent cells to a possible role in the induction or suppression of the response of these cells to antigenic stimulation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5059(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization of Antigen Specific T-Cell Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Singer, M.D.	Clinical Associate	IB NCI
OTHER:	H. B. Dickler, M.D.	Senior Investigator	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

Antigen specific T-cell factors are most likely the solubilized T-cell antigen receptor, since it is unlikely that the T-cell has two different antigen specific molecules, the factor and a different receptor. Therefore, antigen specific T-cell factors offer a direct way to study the structure of the T-cell antigen receptor. In addition, the immunoregulatory functions of these factors provide a way of studying specific B-cell activation and T-B cooperation.

Project Description:

Objectives: The three major objectives of this project are: (1) the confirmation of the existence of antigen specific T-cell factors, (2) the physical characterization of such factors, and (3) the study of the immunoregulatory function of antigen specific T-cell factors in T-B and T-T cooperation.

Methods Employed: Thymocytes from C57BL/10 mice educated to TGAL are cultured with TGAL for 6-8 hours under serum-free conditions. The supernatants from these cultures contain an antigen specific T-cell product which can replace T-cells in an adoptive transfer experiment. The biologic activity of this factor is assayed by adoptively transferring into a syngeneic animal either bone marrow cells and TGAL, bone marrow cells plus educated thymocytes and TGAL, or bone marrow cells plus the supernatant factor and TGAL. The development of specific antibody producing cells in the spleens of the transferred animals is assayed by the Jerne plaque technique. The following methods are used to characterize the factor:

(a) the use of genetically different strains of mice to produce and respond to the factor,
(b) column purification of the factor,
(c) titration of varying amounts of factor with B-cells,
(d) gel filtration to determine molecular weight,
(e) absorption of factor by anti-Ia, antigen, anti-immunoglobulin, and anti-idiotype immunoadsorbents.

Major Findings: TGAL specific T-cell factor can be obtained from educated thymocytes cultured with TGAL under serum-free conditions for 6-8 hours. This factor can cooperate with normal bone marrow cells and TGAL to induce the development of cells which secrete specific anti-TGAL antibody in the absence of T cells. The factor produced by TGAL educated thymocytes is antigen specific and does not cooperate with B-cells to form anti-SRBC antibodies.

Significance to Biomedical Research and the Program of the Institute: T-cells are one type of lymphoid cell involved in tumor recognition and destruction, yet the T-cell antigen recognition system is poorly understood. Greater insight into the structure and function of the T-cell antigen receptor will likely lead to a deeper understanding of the roles T-cells and their products play in tumor immunology. Furthermore, antigen specific T-cell factors can function as immunoregulatory molecules either enhancing or suppressing antigen specific immune responses. They offer a potentially potent tool for manipulating the immune response against specific tumor targets.

Proposed Course of Project: The project will be directed toward developing an antigen specific T-cell factor against Staphylococcal Nuclease. The immune response to Staphylococcal Nuclease is also under Ir gene control and appears to be controlled by the Ir-b subregion. We then hope to determine several structural and functional characteristics of this factor.

Structurally, we hope to determine (a) whether the antigen specific T-cell factor bears the same idiotype as immunoglobulin (the antigen specific B-cell product), (b) if the Ia antigens constitute a portion of the factor, and (c) what molecules bind the factor to the B-cell surface, and specifically whether they are related to either the Ia antigens and/or the Fc receptor. Functionally, we would like to determine (a) whether the factor, in high concentration, will lose its antigen specificity and become nonspecific, and (b) if the antigen specific T-cell enhancing and suppressing factors are identical but reflect different biologic functions of the factor under different conditions.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5060(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiological Role of Lymphocyte Surface Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Henkart, Ph.D.	Senior Investigator	IB NCI
OTHER:	J. L. Ryan, M.D., Ph.D.	Clinical Associate	IB NCI
	S. Ben-Sasson, Ph.D.	Visiting Fellow	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

The function of Fc receptors in mouse spleen lymphocytes was studied by examining the effect of immobilized antigen-antibody complexes on DNA synthesis induced by various mitogens and on antibody synthesis induced by antigen and B cell mitogens. It was found that the mitogenic stimulation of cells bearing Fc receptors was dramatically inhibited by immobilized antigen-antibody complexes. This inhibition was Fc specific and did not occur when the cells were presented with soluble antigen-antibody complexes. This effect was seen with LPS, lipid A, PPD, poly I:C, cGMP and Con A. However, the mitogenic effect of PHA, which stimulates a subset of T cells not possessing the Fc receptor, was not depressed by immobilized complexes. The LPS mitogenic effect was inhibited in both normal and congenitally athymic mice. A similar inhibition of the polyclonal production of antibody forming cells by LPS was observed when the cultures were carried out in the absence of xenogeneic serum. These results suggest that the Fc receptor may function to generate an intracellular "off" signal for proliferation and differentiation and may play a role in the regulation of the immune response.

Project Description:

Objective: To define the physiological function of the Fc receptor and other lymphocyte surface receptors.

Methods Employed: Mouse splenic lymphocytes were cultured in microtiter plates coated with TNP protein or anti-TNP-TNP-protein as described previously. The mitogenic experiments were pulsed with ^{3}H-thymidine after 48 hours, and one day later harvested and the thymidine incorporated into DNA measured. The antibody-forming cell experiments were assayed after 4-5 days of culture and were assayed by looking for the production of "plaques" as originally described by Jerne. Cyclic nucleotide levels were measured by radioimmunoassay.

Major Findings: The function of Fc receptors in mouse spleen lymphocytes was studied by examining the effect of immunobilized antigen-antibody complexes on DNA synthesis induced by various mitogens and on antibody synthesis induced by antigen and B cell mitogens. It was found that the mitogenic stimulation of cells bearing Fc receptors was dramatically inhibited by immobilized antigen-antibody complexes. This inhibition was Fc specific and did not occur when the cells were presented with soluble antigen-antibody complexes. This effect was seen with LPS, lipid A, PPD, poly I:C, cGMP and Con A. However, the mitogenic effect of PHA, which stimulates a subset of T cells not possessing the Fc receptor, was not depressed by immobilized complexes. The LPS mitogenic effect was inhibited in both normal and congenitally athymic mice. A similar inhibition of the polyclonal production of antibody forming cells by LPS was observed when the cultures were carried out in the absence of xenogeneic serum. These results suggest that the Fc receptor may function to generate an intracellular "off" signal for proliferation and differentiation and may play a role in the regulation of the immune response. The cAMP levels were measured at various times after contact of the cells with immobilized complexes. It appears that these levels are increased approximately 1.5-3x during the first 3 hours after contact, suggesting that this "second messenger" may be playing a key role in mediating the observed inhibition.

Significance to Biomedical Research and the Program of the Institute: The physiological role of the lymphocyte Fc receptor is unknown. These results suggest that it may play a regulatory role to inhibit antibody synthesis once sufficient antibody has been produced to form complexes.

Proposed Course of the Project: We need to further confirm the cAMp level measurements. We will also attempt to measure cGMP levels by a parallel radioimmunoassay. Preliminary experiments are also under way to examine the role of cell-cell interaction in such basic immunological phenomena as B cell mitogenesis and MLC reactions with normal and tumor cells.

Publications:

Ryan, J. S., Arbeit, R. D., Dickler, H. B. and Henkart, P. A.: Fc receptor mediated inhibition of B lymphocyte mitogenesis. In Seligmann, M. (Ed.): Membrane Receptors of Lymphocytes. Amsterdam, North-Holland Publishing Co., 1975, pp. 389-392.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI ZO1 CBD 5061(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Killer Lymphocyte Adhesion to Target Surfaces

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Henkart, Ph.D.	Senior Investigator	IB NCI
OTHER:	E. Alexander, M.D., Ph.D.	Investigator	IB NCI
	H. B. Dickler, M.D.	Senior Investigator	IB NCI
	R. D. Arbeit, M.D.	Clinical Associate	IB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the interaction of human lymphocytes bearing the Fc receptor with surfaces coated by antigen-antibody complexes. This interaction is a model for the binding of killer lymphocytes to antibody-coated target cells which leads to the death of the target cell. A morphological alteration in the lymphocytes associated with this interaction was found to be inhibited by drugs affecting energy metabolism, microtubules, microfilaments and intracellular cAMP levels. There is an excellent quantitative correlation of the inhibition of these drugs of the visible flattening of these cells and the corresponding drug inhibition of the killing mediated by these cells. This implies that a similar flattening may be an important aspect of the killing process. An EM study showed, unexpectedly, that all of these cells bearing the Fc receptor contained bundles of macrotubules in their cytoplasm; several lines of evidence suggest that they are composed of tubulin and may play a role in uropod formation. The adhesion process in these cells is accompanied by a 4-5 fold increase in glucose oxidation to CO_2 and in lactate production, while increases in protein and DNA synthesis do not occur.

Project Description

Objectives: Our overall objective is to define the mechanism by which lymphocytes destroy foreign cells. To this end we have dissected the killing process into discrete steps which can be studied independently. In this project we have studied the initial phases of lymphocyte attachment to the target cell.

Methods Employed: The Fc receptor was detected by forming soluble TNP antigen-antibody complexes in antigen excess, allowing them to bind to a lymphocyte suspension, and detecting the bound antibody by indirect immunoflourescence. As previously described, plastic surfaces coated with antigen-antibody complexes were created by TNP modifying adsorbed proteins followed by coating with anti-TNP. The human lymphocytes bearing Fc receptors adhere to these surfaces and are observed by phase microscopy, or are fixed and examined by the EM.

Major Findings: 1) The method of indirect fluorescence after lymphocyte binding of soluble antigen-antibody complexes has been used successfully to detect the Fc receptor on human and mouse lymphocytes. No binding was detected using soluble complexes of antigen and $F(ab')_2$ antibody. A subfraction of mouse thymus cells and splenic T cells were shown to contain an Fc receptor which requires a higher concentration of soluble complexes for detection (collaboration with Drs. Dickler and Arbeit). 2) The use of plastic surfaces coated by antigen-antibody complexes was studied as a model for the lymphocyte binding to antibody-coated target cells. A morphological alteration in the lymphocytes, consisting of flattening and elongation, was observed to accompany the adhesion of lymphocytes to the target membranes. The drugs vinblastine and cytochalasin B, as well as several drugs raising intracellular cAMP levels, cause an inhibition of these shape changes, as does cold temperature or EDTA in the medium. The inhibition effects closely parallel the inhibition of the killing of antibody-coated target cells by these lymphocytes. Azide and antimycin A, both mitochondrial inhibitors, inhibit the elongation process but not flattening or killing. These results suggest that a process similar to the flattening may be required for killing. This adhesion process in these cells is accompanied by a 4-5 fold increase in glucose oxidation to CO_2 and in lactate production, while increases in protein and DNA synthesis do not occur. 3) The electron microscope shows that the adherent cells possess large numbers of microfilaments and microtubules near their contact with the substrate. These organelles were not present in significant numbers prior to the shape change and appear to have polymerized from monomer. All adherent cells were seen to contain several organelles we have termed macrotubule bundles. These were shown to be present in all normal human Fc receptor-bearing lymphocytes in suspension, and their morphology and drug sensitivity suggest that they are composed of tubulin, the microtubule protein (collaboration with Dr. M. Henkart).

Significance to Biomedical Research and the Program of the Institute:
1) The introduction of a simple and reliable marker for the Fc receptor allows the measurement of this marker on cells from patients with immunodeficiency and lymphoproliferative disorders. This should enhance our understanding of these clinical conditions, as well as allow a more general measurement of this marker in studies aimed at understanding lymphocyte differentiation.
2) The notion of a killer lymphocyte activation process subsequent to binding of the target cell is given experimental support by these studies. Such an activation has been hypothesized since it is clear that mere binding of a killer to a target cell is not adequate to insure the latter's death. This activation appears to be reflected in a flattening of the lymphocyte as well as the increased glucose metabolism. The effect of various drugs on killing may be exerted at this step rather than in the synthesis of a lytic molecule as has been previously interpreted. These studies provide a unique insight into a step of the killing process which hitherto has not been studied.
3) The cell biology of the activation process and its associated shape changes provide an in-depth look at an important aspect of the killing process. Furthermore, the finding of a characteristic cytoplasmic organelle in Fc receptor-bearing cells provides an important marker of lymphocyte differentiation. It has already been used by pathologists without recognition of its relationship to the Fc receptor. The present findings put these findings of macrotubule bundles in various patients' lymphocytes in a new perspective.

Proposed Course of the Project: The intracellular levels of cyclic nucleotides of the adherent cells will be examined before and after attachment to the substrate to see if cAMP or cGMP may be a "second messenger" in this system. Indirect immunofluorescent staining with antitubulin will be used to attempt to answer the question of whether or not macrotubule bundles are composed of tubulin.

Publications:

Arbeit, R. A., Henkart, P. A. and Dickler, H. B.: Lymphocyte binding of heat-aggregated and antigen complexed immunoglobulin. In Bloom, B. and David, J. (Eds.): In Vitro Methods in Cell Mediated Immunity. New York, Academic Press, in press.

Alexander, E. and Henkart, P.: The adherence of human Fc receptor-bearing lymphocytes to antigen-antibody complexes. II. Morphologic alterations induced by the substrate. J. Exp. Med. 143: 329-347, 1976.

Abelson, L., Henkart, P. and Mann, D.: Typing for specific B lymphocyte antigens. In Yunis, E. (Ed.): Manual of Clinical Immunology New York, Academic Press, in press.

Mann, D. L., Abelson, L., Henkart, P., Harris, S. D. and Amos, D. B.: Specific human B lymphocyte alloantigens (L-B) linked to HL-A. Proc. Natl. Acad. Sci. 72: 5103-5106, 1975.

Mann, D. L., Abelson, L., Henkart, P., Harris, S. and Amos, D. B.: Serologic detection of B lymphocyte antigens. In Kissmeyer-Nielsen, F. (Ed.) Histocompatibility Testing 1975. Copenhagen, Munksgaard, 1975, pp. 705-705.

Henkart, M. P. and Henkart, P. A.: The macrotubule bundle: A cytoplasmic organelle characteristic of normal human blood lymphocytes bearing the Fc receptor. Proc. Natl. Acad. Sci., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI Z01 CBD 5062(c)-01 I

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Application of Rapid Flow Microfluorometry to Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Wunderlich, M.D.	Senior Investigator	IB NCI
OTHER:	S. O. Sharrow	Chemist	IB NCI
	W. E. Paul, M.D.	Chief, Lab. of Immunology	LI NIAID
	T. M. Chused, M.D.	Medical Officer (Research)	LMI NIDR
	D. H. Sachs, M.D.	Head, Transplantation Biology Section	IB NCI

COOPERATING UNITS (if any)

CDR Irwin Scher, M.D., Richard Wistar, Jr., M.D., Ph.D., Aftab Ahmed, Ph.D., Dept. of Clinical and Experimental Immunology, Naval Medical Research Institute, Bethesda, MD. Graham L. Campbell, Ph.D. The Wistar Inst. of Anat. and Biology, Philadelphia, PA.

LAB/BRANCH Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

SUMMARY OF WORK (200 words or less - underline keywords)

Using rapid flow microfluorometry for analysis and sorting of cells, a subclass of murine B lymphocytes has been identified which has a critical role in humoral immune responses. The same technical approach has been used to classify murine brain cells.

PHS-6040

Project Description:

Objectives: The objective of this project is to utilize a fluorescent activated cell sorter to aid important studies which would be impossible or extremely difficult to pursue without such technical support. Since this instrument and its supporting personnel serve as a regional resource, the individual projects can differ considerably.

Methods Employed: Cells have been both analyzed and separated by rapid flow microfluorometry using a fluorescent activated cell sorter (FACS). These studies involve preliminary treatment of suspensions of viable, dispersed cells with purified, well-characterized fluorescent reagents.

Major Findings: Use of a fluorescent activated cell sorter (FACS) has provided nearly all of the data for 3 independent studies which are near completion. a) By using the analytical capabilities of FACS, Scher et al. have identified a subpopulation of murine B lymphocytes with low to intermediate levels of surface immunoglobulin (Ig). This subpopulation is absent in a particular inbred strain of mice with an X-linked deficit in humoral responses. This subpopulation is present in normal adult mouse spleens but not in marrow. It is absent in normal neonatal mice. These cells appear to be a mature subpopulation of B lymphocytes, which are necessary for normal responses to T independent antigens and B cell mitogens. Several analogies not detailed here suggest they are equivalent to IgD bearing lymphocytes in humans. b) Further studies of humoral immune responses have been done with Dr. Chused. This work has shown that NZB mice, a strain with pronounced tendency for autoimmune disease, have a marked increase in B lymphocytes with low levels of surface Ig. These findings stand in contrast to previous reports that NZB mice have increased numbers of cells lacking surface Ig. Apparently, earlier workers using visual fluorescent microscopy did not detect the low levels of cell surface Ig detected by FACS. c) Campbell and colleagues are the first to have used FACS to analyze central nervous system cells. Ten day old mouse brains were dispersed into viable, single cell suspensions using mechanical and enzymatic treatments followed by separation of cells on discontinuous BSA gradients. These cells were successfully labeled with fluorescent anti-corpus callosum reagents and separated by FACS. Sufficient quantities of viable, separated cells were available for biochemical analysis. Cells labeled by the antiserum had comparatively high levels of CNP hydrolase activity and S-100 and GFA proteins.

Significance to Biomedical Research and the Program of the Institute: Investigating the nature of the humoral immune response with FACS is important to tumor immunology insofar as this response may contribute to rejection of leukemic cells and may block cellular immune activity. This work has led to identification of a new subclass of B lymphocytes with a critical role in humoral immune responses. A separate investigation of central nervous system cells with FACS has resulted in model procedures for similar studies of any solid tissue (such as tumor) by use of rapid flow microfluorometry.

Proposed Course of Project: As in the past the fluorescent activated cell sorter will serve as a regional resource.

Publications:

Fathman, C.G., Cone, J.L., Sharrow, S.O., Tyrer, H. and Sachs, D.H.: Ia alloantigen(s) detected on thymocytes using a fluorescence activated cell sorter. J. Immunol. 115:584-589, 1975.

Scher, I., Ahmed, A., Sharrow, S.O., Steinberg, A.D. and Paul, W.E.: Genetic control of B-lymphocyte function in the CBA/N mouse strain: A model for examining the mechanism of B-lymphocyte activation. In Role of Mitogens in Immunobiology (J. Oppenheim and D. Rosenstreich, Eds.). Academic Press, New York, in press.

Scher, I., Sharrow, S.O., Wistar, R., and Paul, W.F.: Density of surface Ig on B cells: Ontogenic studies of a population of splenic B cells in normal mice and mice with an X-linked B-lymphocyte immune defect. In Proceedings of 10th Leucocyte Culture Conference Amsterdam, Netherlands, in press.

ANNUAL REPORT OF THE LABORATORY OF IMMUNODIAGNOSIS,

NATIONAL CANCER INSTITUTE

July 1, 1975 - June 30, 1976

This report summarizes the activities of the Laboratory of Immunodiagnosis for its first full year, since its initiation in December, 1974. During this year, there have been several substantial changes in the composition and activities of the Laboratory. There has been a considerable increase in the number of professional personnel, consisting mainly of postdoctoral fellows and guest workers. The immunochemical and serum diagnostic markers areas have been strengthened considerably by the transfer of Dr. K. Robert McIntire into this laboratory from the Laboratory of Cell Biology, and by the addition of Drs. James Braatz and A.K. Ng, who have good biochemical backgrounds. There has been a major increase in the amount of research activity performed directly within the laboratory related to clinical tumor immunology, with the addition of several investigators who have strong clinical backgrounds and interests. The loss of Dr. Robert K. Oldham from the Laboratory in December 1975 (who left to become Chief of Medical Oncology at Vanderbilt University Medical School) has been well offset by the assumption of responsibilities for clinical investigations by Dr. Guy Bonnard, who has considerable laboratory research experience as well as clinical training. In regard to specific areas of research, there has been a substantial increase in studies related to characterization of the effector cells in various assays by analysis of cell surface markers, in investigations on the in vivo relevance of various in vitro observations, and in the careful immunologic monitoring of patients with cancer.

In addition to the research activities of the Laboratory which are summarized below, Drs. Herberman and McIntire are extensively involved in the National Cancer Program activities related to immunodiagnosis of cancer. Both serve on the Committee on Immunodiagnosis of Cancer and other contract review committees, and serve as Project Officers on contracts related to immunodiagnosis. Dr. Herberman is the main focus within the National Cancer Institute for problems related to immunodiagnosis, and he has been particularly involved with evaluation of many potentially useful serum immunodiagnostic tests, by the use of coded serum panels provided to investigators from the NCI-Mayo Clinic Serum Bank.

Because of the limited space and technical staff which have been available to the Laboratory, its activities are substantially augmented by support contracts with Litton-Bionetics, Inc., Kensington, Md., and with George Washington University Medical Center, Washington, D.C. These permit more extensive studies of in vivo tumor immunity in experimental mice and rats, in vitro assays of immunity of cancer patients by established procedures, and large scale separations of human tumor associated antigens. These support contract projects are performed in very close association with the intramural activities of the Laboratory.

The research activities of the Laboratory of Immunodiagnosis can be divided into three main areas: Cell-mediated immunity studies in animal tumor systems, cell-mediated immunity studies in man, and immunochemical studies of tumor associated antigens. The main emphasis in each of these areas is on the application of the research information to the diagnosis of cancer and monitoring of disease in tumor bearing individuals.

Some highlights of achievements in the past year:

I. Cell-Mediated Immunity Studies in Animal Tumor Systems.

Extensive studies have been continued on the cell-mediated immune response of C57BL/6 mice to inoculation with murine sarcoma virus (MSV). The ^{51}Cr release cytotoxicity assay has been utilized in many of these experiments. Previously it has been demonstrated that cytotoxic reactivity, as measured by the 4 hour ^{51}Cr release cytotoxicity assay, of spleen cells from C57BL/6 mice injected with MSV 14 days earlier was T cell-dependent and was directed against endogenous type C virus antigen, designated MEV-SA-1. At first there appeared to be an association between the H-2 antigen and the MEV-SA-1 since it was mostly syngeneic targets that were lysed by the effector cells. However, if the allogeneic target cells were grown *in vitro*, this antigen could be detected by the 4 hour inhibition of lymphocyte-mediated ^{51}Cr release cytotoxicity assay. In addition different endogenous type C viruses isolated from these tumor lines could cause expression of MEV-SA-1 on feral mouse cells and also on xenogeneic rabbit cells as detected by the inhibition assay. Since most of the cross-reacting antigens on allogeneic targets had been detected by use of the inhibition assay, and particularly since Zinkernagel, Shearer and others have reported that T cell mediated cytotoxic reactions are directed against modified H-2 antigens and do not affect allogeneic targets, we have examined the specificity in this system more extensively, using the more sensitive 18 hour ^{51}Cr release assay. Most of the allogeneic targets which were positive in the inhibition assay after they had been cultured *in vitro* were also positive in the direct 18 hour test although the levels of cytotoxic reactivity were generally not as high as against the syngeneic targets. Treatment with anti-Θ plus complement indicated that the cytotoxicity against tumor antigens on the allogeneic targets was also T cell-dependent. Studies of the specificity of migration inhibition reactions (see below) in this system, which were T cell dependent, also showed reactivity against allogeneic tumor cells. Thus it appears from our data that the close association of T cell mediated immunity with H2 antigens has been over-generalized, and that significant T cell immune responses can be directed against tumor associated antigens on allogeneic as well as on syngeneic tumor cells.

An apparent paradox in the MSV tumor system and other syngeneic systems has been the short-lived cytotoxic cellular immune response, in contrast to the long lasting *in vivo* resistance to tumor challenge. We have demonstrated

that challenge with tumor cells carrying the appropriate antigens, even many months after primary inoculation with MSV, resulted in rapid appearance of a secondary cytotoxic response. Further studies have shown that the ability to detect cytotoxic reactivity was a function of the length of time between primary and secondary challenge, the site at which the animals were challenged, the site from which effector cells were harvested, the length of time between the secondary challenge and the harvest of the effector cells, and the size of the challenge inoculum. Each of these variables acted independently to influence the kinetics of the secondary cytotoxic response, making it difficult a priori to determine the optimum time for detecting a secondary cytotoxic response.

As described last year, lymphoid cells from many normal mice and rats, of a variety of inbred strains, were found to have reactivity, in the 4 hour ^{51}Cr release cytotoxicity assay, against several syngeneic and allogeneic tumors. During the past year, extensive studies have been performed to further characterize this phenomenon. Natural cytotoxic reactivity was found in other, long term assays of cytotoxicity, including the $^{125}IUdR$ release and ^{3}H-proline release assays. Susceptibility to damage by natural killer cells was not limited to lymphomas; a wide variety of carcinomas and sarcomas were also affected. In addition, normal thymus cells of C57BL/6 and BALB/6 mice were also lysed. Very high cytotoxic reactivity was detected in athymic, nude mice and in CBA/N mice and low activity occurred in A strain mice. Although we and others had previously failed to detect any identifying surface markers on the natural effector cells, several pieces of evidence have indicated that these cells may be precursors of T cells. An inverse relationship between natural reactivity and thymus function was found. Incubation of nude spleen cells in vitro with human thymosin caused a decrease in cytotoxicity, whereas this treatment had no effect on the reactivity of cytotoxic T cells of mice immunized against MSV. Treatment of nude spleen cells with anti-θ serum plus complement has caused up to 50% decrease in reactivity. Based on parallel data on the characteristics of the human natural killer cell, further studies were performed to determine whether the mouse natural killer cells have receptors for immunoglobulins. Pretreatment of cells on monolayers of complexes of sheep erythrocytes and antibodies to sheep cells (EA) resulted in partial decrease in total cytotoxic reactivity. Also, addition of EA to the cytotoxicity assay consistently reduced the level of lysis. These data suggest that both the mouse and the human natural cytotoxic cells have Fc receptors and that they may be in the T cell lineage.

Since previous studies on the specificity of the natural cytotoxicity indicated that specific antigens, possibly endogenous type C virus associated antigens, were being detected, and since our studies indicated that it was possible to boost the levels of immune T cell cytotoxicity by rechallenge with the appropriate antigens, we initiated studies to determine whether the levels of natural reactivity could also be boosted. Challenge of either nude mice or conventional BALB/c mice with a variety of mouse tumor cells and

thymus cells, known to contain antigens involved in natural cytotoxicity, resulted in a rapid and consistent rise in cytotoxic reactivity, reaching a peak at 3-4 days. Inoculation of other materials, including mouse erythrocytes and rat and human tumor cells had no such effect. The effector cells mediating the augmented cytotoxicity had the same characteristics as those in nonstimulated mice.

The natural reactivity of mice was found to be relatively radioresistant, but it fell rapidly to low levels after treatment of mice with cyclophosphamide. These and some other characteristics of the natural cytotoxic reactivity have suggested an analogy with the Hh system mediating resistance to bone marrow transplants. Studies are now in progress to determine whether the mechanisms for each are the same.

The most important issue related to the natural cytotoxicity is its possible in vivo role in resistance against tumor growth. A series of experiments have been initiated to examine this. Several mouse tumors have been found to grow less well in nude mice than in normal mice. Each such tumor that we have examined was found to be very susceptible to natural cytotoxicity. Some susceptible tumors were also found to grow less well in 6-8 week old mice than in mice older than 3 months, which correlates well with the peak of natural reactivity in young mice. As seen with their effects on natural reactivity, X-irradiation of nude mice had no deleterious effect on resistance of nude mice to tumor growth, whereas treatment with cyclophosphamide caused enhanced tumor growth.

Rats immune to the Gross virus-induced lymphoma, (C58NT)D, and mice immunized by MSV have been previously shown in this laboratory to have lymphoproliferative responses to tumor cells bearing the relevant antigens. However, these responses were quite variable and difficult to detect, particularly in tumor-bearing individuals. Earlier work in this laboratory indicated that suppressor macrophages in the spleens or peritoneal cavity of mice could interfere with these responses, and appeared to be one major explanation for these difficulties. Further studies have been performed to understand this phenomenon, and also to see if similar cells were involved in the rat tumor system as in mice. The suppressor macrophages of mice were found to inhibit not only proliferative responses, but also the in vitro and the in vivo generation of cytotoxic T cells. These effects appeared to be dependent on intact cells. Initial experiments suggested that supernatant factors from active macrophages could cause inhibition of ^{3}H-thymidine incorporation by lymphocytes, but this was found to be related only to interference of labelled thymidine uptake, presumably by thymidine released from the macrophages, and there were no effects on actual proliferation of the lymphocytes.

Based on these studies of suppressor cells in mice, major improvements have been made in the ability to detect specific lymphoproliferative responses of immune rats to (C58NT)D tumor cells. It was found that tumor bearing rats had increased levels of suppressor cells in their spleens, but that, in contrast to mice, even normal rats and immune rats had considerable suppressor activity. The suppressor cells could be removed by passage over

rayon adherence columns or by removal of phagocytic cells by treatment with carbonyl iron plus magnet. Depletion of T cells with a specific anti-T cell serum plus complement did not affect the suppressor activity. Therefore, as in the mouse, the suppressor cells appear to be macrophages. Removal of the suppressor cells, and performance of the cultures in the presence of 2-mercaptoethanol, have resulted in reproducibly high levels of lymphoproliferative responses both in immune and tumor-bearing rats.

The suppressor macrophages have also been found to have strong inhibitory effects on the proliferation of many tumor cells in vitro. In both the mouse and the rat, the growth inhibitory cells appear to be the same as the suppressor cells. The growth inhibition assay has provided a rapid and simple assay for the levels of suppressor cell activity in individual animals.

Experiments were performed to determine whether the growth inhibitory and suppressor activities of macrophages had significant effects in vivo or whether they could only be detected as in vitro phenomena. When MSV tumor bearing animals were injected with the allogeneic P-815 mastocytoma, the primary cytotoxic response to this tumor was often decreased when compared to normal controls, presumably due to the suppressor cells which were induced by the tumor. In addition, if MSV immune animals were challenged with X-irradiated RBL-5 tumor cells mixed with suppressor spleen cells from animals injected with C.p. there was an inhibition of the secondary cytotoxic response. Similarily, if animals were injected with C.p. and then injected with MSV, the level of cytotoxic reactivity was reduced at day 14 when compared to controls. Macrophage mediated growth inhibitory activity could also be detected in vivo. Spleen cells from animals inoculated 14 days earlier with MSV were treated with anti-Θ plus complement to eliminate the cytotoxic T cells. These cell suspensions when mixed with RBL-5 lymphoma cells and inoculated subcutaneously into normal, syngeneic mice inhibited the tumor growth whereas normal spleen cells did not.

Thus, both in vitro and in vivo, macrophages from tumor bearing mice and rats or C.p. treated animals can have both immunosuppressive and antitumor effects. It remains to be determined how these functions influence tumor growth under various natural conditions.

In addition to suppressor macrophages, it was found that some transplantable tumor cells themselves could inhibit lymphoproliferative reactions. In both mouse and rat tumors, this inhibitory activity was found to be due to contamination by parvoviruses. The (C58NT)D rat lymphoma and 13762, a rat mammary adenocarcinoma which is widely used in tumor immunity studies, were found to be contaminated with Kilham rat virus (KRV). This virus has strong inhibitory activity on lymphoproliferative responses to mitogens and antigens, presumably because of a selective cytopathic effect on proliferating T cells. Tissue cultured (C58NT)D cells, which lack detectable KRV, have no inhibitory activity. Treatment of infected tumor cells or KRV with anti-KRV antiserum removed inhibitory activity. EL-4 mouse lymphoma cells, and several other mouse transplantable tumors have been found to be contaminated with minute virus of mice (MVM). As with KRV, MVM has strong

inhibitory activity on lymphoproliferative responses in mixed lymphocyte cultures. Treatment of infected tumor cells or MVM with anti-MVM antiserum removed inhibitory activity. The effects of both parvoviruses were selective in their effects on immune responses, having no effect on in vitro induction of T cell dependent antibody responses.

On the assumption that the most important immunological responses against the tumor should be occurring at the site of tumor growth, we have initiated a study to evaluate the ability of host cells within primary MSV-induced tumors to function as effector cells in vitro. Host cells were obtained by enzymatic disaggregation of the tumor and fractionated by sedimentation velocity at unit gravity on a Ficoll gradient. T lymphocytes have been identified in the tumors by their surface markers as detected by indirect immunofluoresence using rabbit anti-mouse brain antiserum and by their functional reactivity as measured by a 4 hour ^{51}Cr release cytotoxicity assay. In addition, macrophages were also found within the MSV tumors as determined by phagocytosis, the presence of an Fc receptor on their surface and functionally by their ability to inhibit the proliferation of lymphoma cells in a growth inhibition assay (GIA) and by their capacity to suppress the PHA induced proliferation of normal spleen cells.

Extensive studies also have been performed using the migration inhibition assays in order to determine the relationship of this assay to the growth and regression of MSV tumors. Both the direct capillary and the indirect agarose assays have been utilized to assay the production of migration inhibition factor (MIF). Using RBL-5 cells as a source of antigen, the kinetics of the response in MSV injected animals as seen in the migration inhibition assay were quite different from those described for the 4 hour ^{51}Cr release cytotoxicity assay. At day 9 after MSV injection, there was a sharp increase in activity which peaked at day 10 and then rapidly declined to very low levels by day 14, the peak of cytotoxic reactivity. A second peak of MIF production was observed at 17-19 days after inoculation and by day 21 the reactivity had subsided. Individual animals that were tested in parallel for MIF production and for cytotoxic activity in the 4 hour ^{51}Cr release cytotoxicity test had strong reactivity in one assay and low activity in the other assay and vice versa. Activity in both assays was T cell-dependent since they could be removed by treatment with anti-Θ plus complement.

A major objective in our studies of cell-mediated immunity to tumor associated antigens is to understand the mechanisms for in vivo resistance to tumor growth. In the (C58NT)D tumor system, we have performed experiments to relate the known in vitro reactivities of lymphocytes to their ability to adoptively transfer resistance against tumor challenge. Systemic administration of lymphocytes from immune rats could protect well against subsequent challenge. Protection was strongest with cells from rats 30-50 days after immunization. This corresponded to peak reactivity in the lymphoproliferative assay with tumor cells, and to strong reactivity to secondary exposure to antigen. Cells taken at 10 days after immunization, at the peak of the primary cytotoxic response, had only weak protective effects. It seems likely that adoptive transfer in this tumor system is mediated primarily by memory cells which are able to circulate well and migrate to the site of

tumor challenge. The ability to adoptively transfer resistance was completely dependent on T cells, being abolished by pretreatment with a specific anti-T cell serum plus complement. Macrophages or other adherent cells were not required for adoptive protection.

Previously we have performed adoptive transfer experiments which demonstrated that immune spleen cells could also transfer immunity against MSV challenge in X-irradiated recipients and that this protection was T cell dependent. These studies were continued in two areas. First, using cryopreserved spleen cells we were able to protect X-irradiated mice against challenge with MSV indicating the feasibility of using this source of cells for repeated prophylaxis or immunotherapy studies. Second, adoptive transfer experiments were performed in which intact tumor cells were employed for challenge, instead of tumor viruses, to eliminate the possibility that virus neutralizing antibody was mediating the protection which we were seeing. Immune spleen cells were capable of transferring resistance to challenge with Rauscher lymphoma, RBL-5 or with an MSV-induced tumor, MSB.

II. Studies of Cell-Mediated Immunity in Man.

Since we had recognized the importance of natural reactivity in various cytotoxicity assays, we reevaluated the specificity of these reactions and studied in detail the characteristics of the effector cells. Considerable efforts were made during this year to analyze our long past experience in tests of cellular cytotoxicity as they relate to the reactivity of normal controls (natural activity), or to the superimposed activity of cancer patients for histologically related tumor target cells (histological type and tumor specific activity). By direct testing of normal peripheral blood lymphocytes against a variety of tumor cell lines, we could confirm that the myeloid line K-562 is a particularly good target yielding high and reproducible results. By competitive inhibition tests in the K-562 system using the various lines as inhibitions, it was found that solid tumor lines generally inhibited quite well, whereas various lymphoid lines, leukemic lymphoblasts, or normal peripheral blood lymphocytes would not inhibit. Thus, the competitive inhibition tests appeared quite powerful in analyzing the specificity of the natural cytotoxicity. Of interest was our finding that some lines from rats and mice inhibited quite well. More recently, we found that 7S EA complexes strongly inhibited the natural cytotoxicity. We found that Chang cell-antibody (rabbit) complexes and plastic bound TNP-anti TNP (rabbit) complexes most effectively inhibited the natural cytotoxicity. Competitive inhibition tests are now being performed in systems with target cells other than K-562 in order to extend these observations.

The inhibition studies with antigen-antibody complexes strongly suggested that the effector cells in the K-562 system bear an Fc receptor, and are at least in this respect similar to the K cells mediating ADCC. We made considerable efforts to test this hypothesis. First, the role of macrophages in the K-562 system was excluded. Then, we were quite successful in removing the natural killer cells by an absorption technique on TNP-anti TNP (rabbit) coated plastic surfaces and by EA monolayers; simultaneous removal of the activity in ADCC against Chang cell antibody complexes

was demonstrated. We would not infer that the Fc receptor bearing natural killer cell is a K cell, nor that the Fc receptor plays any role in the binding to the target cell. Indeed, we have preliminary evidence to the contrary, in as much as various enzyme treatments had disparate effects on ADCC activity and on natural cytotoxicity. Also there is so far no evidence that an antibody is required for occurrence of natural cytotoxicity. Studies were also performed to examine previous reports that the natural cytotoxic cells contained complement receptors and could be depleted by EAC rosettes. When 19S EAC was used, only minor depletion was observed indicating that most of the natural effector cells lack complement receptors. The very strong depletion of the natural cytotoxicity by antigen-antibody complexes is of utmost practical importance since it might allow testing for a remaining superimposed cytotoxicity of a different nature, namely by immune, differentiated T cells.

A considerable emphasis has been placed on evaluation of cellular immune competence in cancer patients. Studies have been performed on rosette formation of human peripheral blood lymphocytes with sheep erythrocytes (E-rosettes). With performance of the assay at 29°C, only 50-55% of normal lymphocytes formed E rosettes, compared to 75-80% at the more optimal temperatures of 4°C. The T cells which form rosettes at 29°C were found to be a separable subpopulation of cells, with different functions than those which only form rosettes at lower temperatures. It was possible, however, to change the relative proportions of these cells by incubation in vitro with human thymosin. This resulted in an increase, by 10-15%, in the percentage of cells from normal individuals forming rosettes at 29°C but had virtually no effect on the percentage of E-rosettes at 4°C. Larger increases in percent 29° rosetting cells were seen with some cancer patients. Since this incubation also caused a decrease in natural cytotoxic reactivity of human lymphocytes against the K-562 tissue culture cell line, this suggests that the state of differentiation of human T cells is a factor in the stability of rosette formation and in some functional activities. The 29° rosetting technique has been very well standardized, and this has allowed its application to the study of the percentage of rosette forming cells in the peripheral blood of cancer patients. The earlier impression that the percentage of 29°C RFC was generally depressed in cancer patients was confirmed, and its significance was enhanced by findings such as the stronger depression in one histological type of lung cancer (anaplastic), as opposed to other types, and that the fall in the proportion of 29°C RFC occurred a few months before clinical recurrence of lung cancer. Therefore, considerable effort is now being made to systematically follow-up patients with this test and assess its value for prognosis and detection of recurrent disease. Studies are also being performed to determine the correlation between % RFC and functional activity in assays of cell-mediated immunity.

It was also clearly shown during this year that many patients with lung and breast cancer have depressed lymphoproliferative responses to the mitogens PHA and Con A, and to alloantigens in mixed lymphocyte cultures. This demonstation was considerably facilitated by the development of the Relative Proliferative Index (RPI) to express the results. This was based on a comparison of the levels of proliferation of cancer patients' lymphocytes to those of normal donors tested at the same time.

A considerable emphasis has continued to be placed on cell-mediated immunity of cancer patients to tumor associated antigens. We continue to measure delayed hypersensitivity reactions of patients to Sephadex G-200 and polyacrylamide gel electrophoresis purified soluble fractions of tumor cell extracts, prepared by Dr. Ariel Hollinshead, George Washington University. We tested such fractions from melanoma, breast, and lung cancers, and found activity to be concentrated in some of the fractions. When fractions of melanoma and breast carcinoma were tested, patients with either disease often reacted to both types of extracts; patients with lung cancer tended to react much less. Patients with breast carcinoma, and only infrequently patients with melanoma or other cancers, tended to react to soluble fractions from a tissue culture line derived from a carcinoma of the breast. This led us to believe that tissue culture cells may be a very good and effective standard source of tumor associated antigens for skin testing.

Further studies with the direct leukocyte migration inhibition assay have also established the value of 3M KCl and more purified extracts of tissue culture lines derived from tumors, as a source of antigen. Repeatedly 3M KCl extracts were made of the MCF-7 breast carcinoma line, which were found to specifically inhibit leukocyte migration of breast cancer patients. Soluble Sephadex G-200 and polyacrylamide gel electrophoresis purified fractions of this MCF-7 line were obtained, and some were found to induce the reaction at very low concentrations of proteins.

We have performed a series of experiments to examine the phenomenon reported by Black of LMI reactivity of breast cancer patients to mouse milk containing mammary tumor virus. Most patients with carcinoma of the breast have been found to react with purified mammary tumor virus prepared either from milk or from tissue culture fluids, and also with gp52, the major surface glycoprotein of the virus. The same materials elicited very little reactivity in normal women or men or in patients with other types of cancer. Studies are being actively pursued to determine the specificity and diagnostic usefulness of these findings.

Another development has been the modification of the microagarose droplet migration assay for the measurement of LMI. Initial experiments were performed with PPD as antigen, using tuberculin skin test positive and negative donors. This assay was found to be very sensitive, detecting reactivity of some skin test positive individuals at picogram or nanogram concentrations of PPD. In addition, this assay reduces the requirement of whole blood to only 5-10 ml and permits much more extensive testing than the more cumbersome capillary tube method. This method has already proven useful in testing multiple soluble fractions from tumor cell extracts, and has detected specific reactivity to nanogram concentrations of some fractions.

Cellular immunity of cancer patients has also been successfully studied with the mixed lymphocyte tumor cell interaction assay. In our studies on selected patients to date, approximately 60% of our tests in lung carcinoma and 40% of our tests in breast carcinoma resulted in clearcut proliferation of lymphocytes to the autologous tumor material. It was found that hypotonic membrane extracts were a particularly good source of antigen in both tumor

systems and these are now used exclusively in a very well standardized microtest. Patients are being studied longitudinally, and correlates are made with the other tests of immunocompetence and tumor immunity, in order to assess the clinical significance of these findings.

Extensive studies have been performed on the cryopreservation of effector cells and of target cells in both the clinical and experimental animal systems. This has proven extremely useful in standardizing the assays and minimizing day-to-day variations. This has also allowed us to rapidly detect changes in the antigenicity and susceptibility to lysis of fresh target cells.

Over the past year we continued to utilize assays mentioned above in monitoring patients with neoplastic disease. This included patients with carcinoma of the lung, Ewing's sarcoma, carcinoma of the breast, melanoma and carcinoma of the colon. In the first three systems this monitoring has been done in patients who are being treated under immunotherapeutic protocols in collaboration with other groups.

III. Immunochemical and Immunodiagnostic Studies of Tumor Associated Antigens.

We are continuing to perform assays for AFP to provide evidence that this marker protein is of great value for following therapy in hepatocellular carcinoma, embryonal carcinoma of the testis and pancreatic carcinoma. We have been able to demonstrate a dramatic response to adriamycin in at least one hepatoma patient where the serum AFP measure proved superior to clinical measures for extent of disease. AFP together with human chorionic gonadotropin (HCG) has proven to be an excellent marker for following the response to chemotherapy in testicular tumors. An important aspect in this disease is the ability to make an early diagnosis of recurrent tumor by noting a rise in either or both markers. There is preliminary evidence to indicate that using these markers will aid significantly in detecting those patients with retroperitoneal node involvement or other metastatic disease following primary orchiectomy.

Both gastric and pancreatic carcinoma have been noted in the past to be associated with CEA, AFP and HCG. We are now engaged in a retrospective study with Dr. V.L.W. Go, (Mayo Clinic) to determine the value of simultaneous measurement of all three markers. At the present time 47 patients with pancreatic cancer have been evaluated and the use of combined markers demonstrates a clear superiority. Twelve patients had only one marker while 6 more had two markers elevated; no patients had elevations of all three.

Studies of a human lung tumor associated antigen have continued. Using ion exchange and affinity chromatography, considerable purification has been achieved. Efforts are now being made to produce more specific antisera and to develop more quantitative assays, especially radioimmunoassay. The separated lung tumor associated antigens are also being tested for reactivity in leukocyte migration inhibition assays.

Efforts are also being directed toward the isolation of surface antigens from mouse virus-induced leukemias. Quantitative humoral and cell-mediated assays suitable for measurement of separated antigens have been set up, and fractionation studies are well underway.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05557-08 LID

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cellular Immune Responses in Experimental Tumor Systems and in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.B. Herberman	Chief, LID	LID NCI
OTHERS:	H.T. Holden	Senior Investigator	LID NCI
	W.H. West	Research Associate	LID NCI
	J.R. Oehler	Guest Worker	LID NCI
	M. Glaser	Visiting Fellow	LID NCI
	D.A. Campbell, Jr.	Guest Worker	LID NCI
	G.D. Bonnard	Visiting Scientist	LID NCI
	S.P. Staal	Expert Consultant	LID NCI
	M.E. Nunn	Biologist	LID NCI

COOPERATING UNITS (if any)
Viral Leukemia and Lymphoma Branch, NCI; Laboratory of Cell Biology, NCI; Laboratory of Viral Diseases, NIAID; Biometric Branch, NCI; Litton Bionetics, Inc.; Purdue University; Institute of Oncology, Warsaw, Poland; Sloan Kettering Institute for Cancer Research; Institute for Cancer Research, Phila., Pa.

LAB/BRANCH
Laboratory of Immunodiagnosis

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.3	5.3	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Natural cell-mediated cytotoxicity against mouse and rat tumor cells has been studied. Normal lymphocytes of many animals are cytotoxic for lymphomas, carcinomas and sarcomas. The natural effector cell lacks easily definable surface markers, but appears to have an Fc receptor and to be an early T cell. The natural cytotoxicity appears to play an important role in in vivo resistance to tumor growth. Lymphoproliferative responses to tumor associated antigens in mice and rats have been detected. These are inhibited by suppressor macrophages, found in increased amounts in tumor-bearing individuals. These cells also can inhibit growth of tumor cells. Lymphoproliferative responses were also markedly inhibited by rodent parvoviruses, frequent contaminants of transplantable tumors. Adoptive transfer of resistance to growth of the rat tumor (C58NT)D was mediated by T cells from immune rats, obtained 30-50 days after immunization. This correlated with the presence of cytotoxic memory cells and of cells responsive in mixed lymphocyte tumor interactions, but not with directly cytotoxic cells. Separable subpopulations of human peripheral blood T lymphocytes formed rosettes with sheep erythrocytes at 29°C or only at 4°C. Thymosin affected the properties of the cells.

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and quantitatiive changes in normal antigens on malignant cells; (2) to detect cellular immune reactions to tumor antigens; (3) to evaluate the diagnostic, prognostic and therapeutic significance of cellular immune responses to tumor antigens; (4) to develop and evaluate immunodiagnostic assays for human cancer; (5) to measure general immunological competence of tumor bearing animals and of cancer patients.

Methods Employed: Lymphoid cells are obtained from tumor-bearing, immune, and normal subjects. A variety of normal and tumor cell lines are maintained in tissue culture. Inbred strains of mice and rats, and transplantable tumors induced by viral, chemical or unknown agents are used in model systems. Cellular immune competence is assayed by rosette formation; lymphocyte stimulation by mitogens, antigens and allogeneic cells; and by cytotoxicity reactions. Cellular immune reactions to tumor antigens are measured by mixed lymphocyte tumor cell cultures, migration inhibition, and lymphocyte-mediated cytotoxicity assay. Tumors and other materials are tested for oncogenic and other viruses. Subpopulations of lymphoid cells are prepared by separation of rosette-forming cells, passage through anti-immunoglobulin columns, adherence columns and plates, and by antiserum treatments.

Major Findings:

I. Natural Cell-Mediated Immunity Against Tumor Cells.

As described last year, lymphoid cells from many normal mice and rats, of a variety of inbred strains, were found to have reactivity, in the 4 hour ^{51}Cr release cytotoxicity assay, against several syngeneic and allogeneic tumors. During the past year, extensive studies have been performed to further characterize this phenomenon. Natural cytotoxic reactivity was found in other, long term assays of cytotoxicity, including the $^{125}IUdR$ release and ^{3}H-proline release assays. Susceptibility to damage by natural killer cells was not limited to lymphomas; a wide variety of carcinomas and sarcomas were also affected. In addition, normal thymus cells of C57BL/6 and BALB/c mice were also lysed. Very high cytotoxic reactivity was detected in athymic, nude mice and in CBA/N mice and low activity occurred in A strain mice. Although we and others had previously failed to detect any identifying surface markers on the natural effector cells, several pieces of evidence have indicated that these cells may be precursors of T cells. An inverse relationship between natural reactivity and thymus function was found. In addition to the high reactivity of nude mice, newborn thymectomy resulted in high reactivity. Implantation of thymus grafts into nude mice caused a prompt fall in reactitity. Incubation of nude spleen cells in vitro with human thymosin caused a decrease in cytotoxicity, whereas this treatment had no effect on the reactivity of cytotoxic T cells of mice immunized against MSV. Treatment of nude spleen

cells with anti-θ serum plus complement has caused up to 50% decrease in reactivity. But, an anti-mouse brain antiserum of Dr. Edward Golub, with known anti-stem cell activity in addition to anti-T cell activity, had no effect. Based on parallel data on the characteristics of the human natural killer cell, further studies were performed to determine whether the mouse natural killer cells have receptors for immunoglobulins. Pretreatment of cells on monolayers of complexes of sheep erythrocytes and antibodies to sheep cells (EA) resulted in partial decrease in total cytotoxic reactivity. Also, addition of EA to the cytotoxicity assay consistently reduced the level of lysis. These data suggest that both the mouse and the human natural cytotoxic cells have Fc receptors and that they may be in the T cell lineage.

Since previous studies on the specificity of the natural cytotoxicity indicated that specific antigens, possibly endogenous type C virus associated antigens, were being detected, and since our studies indicated that it was possible to boost the levels of immune T cell cytotoxicity by rechallenge with the appropriate antigens, we initiated studies to determine whether the levels of natural reactivity could also be boosted. Challenge of either mude mice or conventional BALB/c mice with a variety of mouse tumor cells and thymus cells, known to contain antigens involved in natural cytotoxicity, resulted in a rapid and consistent rise in cytotoxic reactivity, reaching a peak at 3-4 days. Inoculation of other materials, including mouse erythrocytes and rat and human tumor cells had no such effect. The effector cells mediating the augmented cytotoxicity had the same characteristics as those in nonstimulated mice.

The natural reactivity of mice was found to be relatively radioresistant, but it fell rapidly to low levels after treatment of mice with cyclophosphamide. These and some other characterisitcs of the natural cytotoxic reactivity have suggested an analogy with the Hh system mediating resistance to bone marrow transplants. Studies are now in progress to determine whether the mechanisms for each are the same.

The most important issue related to the natural cytotoxicity is its possible *in vivo* role in resistance against tumor growth. A series of experiments have been initiated to examine this. Several mouse tumors have been found to grow less well in nude mice than in normal mice. Each such tumor that we have examined was found to be very susceptable to natural cytotoxicity. Some susceptible tumors were also found to grow less well in 6-8 week old mice than in mice older than 3 months, which correlates well with the peak of natural reactivity in young mice. As seen with their effects on natural reactivity, X-radiation of nude mice had no deleterious effect on resistance of nude mice to tumor growth, whereas treatment with cyclophosphamide caused enhanced tumor growth. Futhermore, it was postulated that if natural reactivity prevented growth of some tumors in nude mice, tumors induced or arising spontaneously in these mice would be insusceptible to natural cytotoxicity. Indeed, several such tumors were found to lack detectable susceptibility to lysis by natural

effector cells, whereas many other tumors arising in conventional mice were susceptible.

II. Lymphoproliferative Responses to Tumor Antigens and Factors Affecting these Responses.

Rats immune to the Gross virus-induced lymphoma,(C58NT)D, and mice immunized by MSV have been shown to have lymphoproliferative responses to tumor cells bearing the relevant antigens. However, these responses were quite variable and difficult to detect, particularly in tumor-bearing individuals. Earlier work in this laboratory indicated that suppressor macrophages in the spleens or peritoneal cavity of mice could interfere with these responses, and appeared to be one major explanation for these difficulties. Further studies have been performed to understand this phenomenon, and also to see if similar cells were involved in the rat tumor system as in mice. The suppressor macrophages of mice were found to inhibit not only proliferative responses, but also the in vitro and the in vivo generation of cytotoxic T cells. These effects appeared to be dependent on intact cells. Initial experiments suggested that supernatant factors from active macrophages could cause inhibition of ^{3}H-thymidine incorporation by lymphocytes, but this was found to be related only to interference of labelled thymidine uptake, presumably by thymidine released from the macrophages, and there were no effects on actual proliferation of the lymphocytes.

Based on these studies of suppressor cells in mice, major improvements have been made in the ability to detect specific lymphoproliferative responses of immune rats to (C58NT)D tumor cells. It was found that tumor bearing rats had increased levels of suppressor cells in their spleens, but that, in contrast to mice, even normal rats and immune rats had considerable suppressor activity. The suppressor cells could be removed by passage over rayon adherence columns or by removal of phagocytic cells by treatment with carbonyl iron plus magnet. Depletion of T cells with a specific anti-T cell serum plus complement did not effect the suppressor activity. Therefore, as in the mouse, the suppressor cells appear to be macrophages. Removal of the suppressor cells, and performance of the cultures in the presence of 2-mercaptoethanol, have resulted in reproducibly high levels of lymphoproliferative responses both in immune and tumor-bearing rats.

The suppressor macrophages have also been found to have strong inhibitory effects on the proliferation of many tumor cells in vitro. In both the mouse and the rat, the growth inhibitory cells appear to be the same as the suppressor cells. The growth inhibition assay has provided a rapid and simple assay for the levels of suppressor cell activity in individual animals.

In addition to suppressor macrophages, it was found that some transplantable tumor cells themselves could inhibit lymphoproliferative reactions. In both mouse and rat tumors, this inhibitory activity was found to be due to contamination by parvoviruses. The (C58NT)D rat lymphoma

and 13762, a rat mammary adenocarcinoma which is widely used in tumor immunity studies, were found to be contaminated with Kilham rat virus (KRV). This virus has strong inhibitory activity on lymphoproliferative responses to mitogens and antigens, presumably because of a selective cytopathic effect on proliferating T cells. Tissue cultured (C58NT)D cells, which lack detectable KRV, have no inhibitory activity. Treatment of infected tumor cells or KRV with anti-KRV antiserum removed inhibitory activity. Of practical interest, X-irradiation of tumor cells had little effect on inhibitory activity, but treatment with mitomycin C virtually abolished inhibitory activity. Such treatment would then allow proliferative responses to infected cells to be studied. EL-4 mouse lymphoma cells, and several other mouse transplantable tumors have been found to be contaminated with minute virus of mice (MVM). As with KRV, MVM has strong inhibitory activity on lymphoproliferative responses in mixed lymphocyte cultures. In contrast, however, neither the infected mouse tumor cells nor the isolated virus have much effect on responses to mitogens. Treatment of infected tumor cells or MVM with anti-MVM antiserum removed inhibitory acitivity. The effects of both parvoviruses were selective in their effects on immune responses, having no effect on in vitro induction of T cell dependent antibody responses.

The identification of immunosuppressive macrophages and viruses emphasize the point that failure to detect immune responses against tumor cells may not indicate lack of immunogenicity of the tumor cells or lack of responsiveness of the host, but may also be due to a variety of inhibitory factors.

III. Specific Depletion of Cytotoxic Effector Cells.

Specific cytotoxicity of MSV immune mouse spleen cells and of alloimmune spleen cells could be depleted by incubation either on monolayer tissue culture cells bearing the relevant antigens or on monolayers of suspension tumor cells, made adherent by coating with poly-L-lysine. This procedure also appears quite promising for the selective depletion of human cytotoxic effector cells.

IV. Adoptive Transfer of Resistance to Tumor Growth.

A major objective in our studies of cell-mediate immunity to tumor associated antigens is to understand the mechanisms for in vivo resistance to tumor growth. In the (C58NT)D tumor system, we have performed experiments to relate the known in vitro reactivities of lymphocytes to their ability to adoptively transfer resistance against tumor challenge. Systemic administration of lymphocytes from immune rats could protect well against subsequent challenge. Protection was strongest with cells from rats 30-50 days after immunization. This corresponded to peak reactivity in the lymphoproliferative assay with tumor cells, and to strong reactivity to secondary exposure to antigen. Cells taken at 10 days after immunization, at the peak of the primary cytotoxic response, had only weak protective effects. It seems likely that adoptive transfer in this tumor system is

mediated primarily by memory cells which are able to circulate well and migrate to the site of tumor challenge. The ability to adoptively transfer resistance was completely dependent on T cells, being abolished by pre-treatment with a specific anti-T cell serum plus complement. Macrophages or other adherent cells were not required for adoptive protection.

V. Rosette Formation and Other Cell-Adherence Phenomena.

As described in the report on project Z01 CBD 08500-08 LID, studies have been performed on rosette formation of human periperal blood lymphocytes with sheep erythrocytes (E-rosettes). With performance of the assay at 29°C, only 50-55% of normal lymphocytes formed E rosettes, compared to 75-80% at the more optimal temperatures of 4°C. The T cells which form rosettes at 29°C were found to be a separable subpopulation of cells, with different functions than those which only form rosettes at lower temperatures. It was possible, however, to change the relative proportions of these cells by incubation in vitro with human thymosin. This resulted in an increase, by 10-15%, in the percentage of cells from normal individuals forming rosettes at 29°C but had virtually no effect on the percentage of E-rosettes at 4°C. Larger increases in percent 29° rosetting cells were seen with some cancer patients. Since this incubation also caused a decrease in natural cytotoxic reactivity of human lymphocytes against the K-562 tissue culture cell line, this suggests that the state of differentiation of human T cells is a factor in the stability of rosette formation and in some functional activities. We have found that the subpopulation of T cells, which can form rosettes at 4°C but not at 29°C is mainly seen in the peripheral blood. Lymphocytes from lymph node cells, tonsils, and pleural effusions formed E-rosettes at 29°C almost to the same extent as at 4°C. In contrast, in two patients with T cell leukemia with cutaneous involvement (one having Sezary cells), the leukemic cells formed very few rosettes at 29°C but rosetted very well at 4°C, suggesting that the leukemia involved the subpopulation of less avid E-rosette forming cells.

Human T Cells were also found to be adherent to, and form rosettes with, human blast cells. In contrast to a report from another laboratory that this adherence was limited to blasts of B cell origin, we have found that rosettes were formed with tissue culture lines derived from T cells as well as B cells, and also with PHA stimulated lymphocytes. Human and rodent lymphocytes have also been found to form rosettes with a variety of tumor cell lines. The possible biologic significance of these interactions is currently under investigation.

Significance to Biomedical Research and the Program of the Institute:

Detection of tumor antigens and of immune reactions to tumor associated antigens is essential for progress in tumor immunology. The sensitive assays of cell-mediated and humoral immunity which have been developed in this laboratory have already proved useful in studying the immune response to animal and human tumors.

The ability of our laboratory to perform multiple assays of cell-mediated immunity to tumor antigens, and to characterize the effector cells, should allow detailed dissection of the role of the immune responses in limiting or preventing tumor growth. These assays may also be useful in immunodiagnosis of cancer and in measuring changes in immunity, in response to chemotherapy or immunotherapy.

Natural cell-mediated immunity may play an important role in immune surveillance against tumors. Understanding of this phenomenon and demonstration of its in vivo significance in mice should be very useful for the understanding of the significance of human natural cell-mediated immunity, which seems to be mediated by the same or very similar types of effector cells.

Proposed Course of Project:

Studies are in progress to further understand the immune response of experimental animals and of patients to tumor associatied antigens. During the next year, the major emphasis will be on determining the in vivo relevance of the various in vitro phenomena that we have been studying. In particular, the role of the natural cytotoxic cells in immunosurveillance and in resistance against tumor growth will be intensively examined. Further studies will also be performed to determine the nature of the natural cytotoxic effector cell, its place in the development of mature T cells, and its relationship to the cells mediating resistance against bone marrow transplants. The in vivo role of the suppressor macrophages will also be intensively studied, to determine whether their immunosuppressive effects or their anti-tumor effects predominate under various circumstances. The possible in vivo effects of the immunosuppressive parvoviruses will also be examined since these ubiquitous contaminants of rodent tumors may have a major influence on the outcome of many studies.

The factors affecting rosette formation between human T cells and sheep erythrocytes will be studied further. In particular, the relationship between T cells rosetting at 29°C and those rosetting only at 4°C will be scrutinized. More generally, the nature of the receptors on lymphocytes for other cells will be explored, particularly in regard to the biological significance of these interactions.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08500-02 LID

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunodiagnosis, Immunotherapy and Immunological Monitoring in Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. D. Bonnard	Visiting Scientist	LID	NCI	(from 12/15/75 on)
	R. K. Oldham	Senior Investigator	LID	NCI	(until 12/15/75)
OTHER:	R. B. Herberman	Chief	LID	NCI	
	J. R. Ortaldo	Biologist	LID	NCI	
	W. H. West	Research Associate	LID	NCI	
	D. A. Campbell	Guest Worker	LID	NCI	
	J. L. Weese	Guest Worker	LID	NCI	
	G. Dunston	Guest Worker	LID	NCI	
	H. D. Kay	Guest Worker	LID	NCI	
	A. Morales	Guest Worker	LID	NCI	
	A. Kibrite	Guest Worker	LID	NCI	

COOPERATING UNITS (if any) Litton-Bionetics, Inc.; G.W. Univ.; R,NCI; M,NCI; Surg,NCI; PO,NCI; I,NCI; VC,NCI; VLL,NCI; U. of Mich.; Howard Univ.; M.D. Anderson Hosp. & Tumor Clin.; Kingston Univ.; Laval Univ.; Georgetown Univ.; Scripps Clin.; Natl. Naval Med. Ctr.; Walter Reed Med. Ctr.; Portsmouth Naval Hosp.; Univ. of So. Carolina; Vanderbilt Univ.

LAB/BRANCH

Laboratory of Immunodiagnosis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.05	7.05	1.00

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical and immunological follow-up of patients with lung and breast cancer, colon carcinoma, Ewing's sarcoma, and melanoma. Adjuvant chemotherapy and immunotherapy with BCG, C. parvum, with or without irradiated allogeneic tumor cells. Skin tests with crude tumor extracts and purified soluble fractions. In vitro assessment of immunocompetence, and of natural cell-mediated reactivity and tumor immune reactivity, by means of short-term cytotoxicity assays, mixed lymphocyte tumor cell culture, and leukocyte migration inhibition. Examination of cells from various lymphoid organs and from the tumor. Reactivity of subpopulations of lymphocytes. Related models: Natural and immune cytotoxic reactivity in the W/Fu rat Gross virus-induced (C58NT)D, and in the F344 rat DMBA-induced 13762 mammary adenocarcinoma models. Cryopreservation of effector-target and inhibitory cells. Effect of X-irradiation on the antigenicity of tumor cells. Comparison of the ^{51}Cr-, ^{3}H-proline-, and ^{125}IUdR cytotoxicity assays. Immunosuppressive activity of tumor cells. Isolation of immunosuppressive Kilham rat virus from these tumors. Immunosuppressive effects of this virus in vivo.

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and to quantitate these as well as normal antigens on tumor cells; (2) to detect cellular immune reactions to tumor antigens; (3) to evaluate the diagnostic, prognostic and therapeutic significance of cellular immune responses to tumor antigens; (4) to develop and evaluate assays for the immunological monitoring and immunodiagnosis of human cancer; (5) to isolate and characterize tumor antigens and other cell surface antigens; (6) to test the reagents developed under (5) in certain *in vitro* assays as well as in delayed hypersensitivity reactions in these tumor systems; (7) to monitor general immunological competence in cancer bearers; (8) to test the therapeutic efficacy of certain immunological stimulants and tumor associated antigens in human tumor systems relative to the clinical state of the host as well as relative to the effect on these assays mentioned above; (9) to evaluate the effects on the immune response of inhibitory phenomena associated with tumor cells and tumor cell growth.

Methods Employed: Tumor cells and normal cells (from lung, breast, Ewing's sarcoma, melanoma, colon) are prepared as single cell suspensions, continuously growing tissue culture lines, hypotonic membrane preparations, saline membrane extracts and/or more purified soluble fractions. Tumor cells can be stored in a viable frozen state. X-irradiated single cell suspensions are used in conjunction with BCG as adjuvant immunotherapy in Ewing's sarcoma and carcinoma of the lung. Immunity *in vivo* is assessed by delayed hypersensitivity reactions (skin tests) to the more purified materials. Natural reactivity and immunity are measured *in vitro* as follows. Lymphocytes are obtained from cancer patients and controls. Relative percentages (patients vs controls) of lymphocytes forming rosettes at 4° C and 29° C, and/or of lymphocytes proliferating to antigens and allo-antigens, and antibody-dependent cellular cytotoxicity in a well characterized system, are measures of general immunocompetence. Natural cell-mediated cytotoxicity against a variety of tissue culture cells are measured in a short term ^{51}Cr-release assay. Selective immunity to tumor associated antigens is detected by proliferative and cytotoxic responses to autologous tumor cells and extracts, and also by the liberation of factors responsible for the inhibition of leukocyte migration. Cells from other lymphoid organs and from the tumor itself are also studied for evidence of immunity. Cell separation techniques are used to detect the subpopulation of lymphocytes responsible for natural cell-mediated cytotoxicity and antibody-dependent cellular cytotoxicity, and for specific acquired immunity.

Major Findings:

I. Role of Adjuvant Immunotherapy:

A. Protocol on Cancer of the Lung.

Results indicated no effects of BCG alone, or BCG plus allogeneic cells in patients with local or disseminated residual tumor burden who were receiving local X-irradiation and combination chemotherapy, and we now plan to enter patients with local residual disease on a new chemo-immunotherapy protocol, which emphasizes the use of high dose methotrexate with or without BCG. It is however too early to determine possible benefits associated with either arm of the earlier protocol for patients who had complete surgical resection of their tumor, and more patients are still being entered on that protocol. Treatment with BCG alone, or BCG plus allogeneic cells, was well tolerated in all patients.

B. Protocol on Cancer of the Breast.

Preliminary results would suggest some beneficial effects of Corynebacterium parvum in this adjuvant chemo-immunotherapy protocol. However, administration of Corynebacterium parvum was not well accepted by the patients, and many withdrew from this arm of the protocol.

C. Protocol on Ewing's Sarcoma.

It is too early to determine the effect of adjuvant immunotherapy in this protocol. We observed one Staphylococcus aureus infection of the injection site, which responded promptly to therapy.

II. In Vivo Delayed Hypersensitivity Testing of Tumor Immunity:

We continue to measure delayed hypersensitivity reactions of patients to Sephadex G-200 and polyacrylamide gel electrophoresis purified soluble fractions of tumor cell extracts, prepared by Dr. Ariel Hollinshead, George Washington University. We tested such fractions from melanoma, breast, and lung cancers, and found activity to be concentrated in some of the fractions. When fractions of melanoma and breast carcinoma were tested, patients with either disease often reacted to both type of extracts; patients with lung cancer tended to react much less. Patients with breast carcinoma, and only infrequently patients with melanoma or other cancers, tended to react to soluble fractions from a tissue culture line derived from a carcinoma of the breast. This led us to believe that tissue culture cells may be a very good and effective standard source of tumor associated antigens for skin testing.

III. In Vitro Testing of Natural Reactivity and Tumor Immunity.

A. Introduction on the Use of Cytotoxicity Assays.

Since we had recognized the importance of natural reactivity in various cytotoxicity assays, we reevaluated the specificity of these reactions and studied in detail the characteristics of the effector cells. Considerable efforts were made during this year to analyze our long past experience in tests of cellular cytotoxicity as they relate to the reactivity of normal controls (natural activity), or to the superimposed activity of cancer patients for histologically related tumor target cells (histological type and tumor specific activity). Efforts have been directed particularly towards testing the reactivity of normals and cancer patients to tumor target cells, including several histologically unrelated target cells, and to show that very little, if any, differences were seen in the levels of cytotoxicity. This conclusion was strengthened by similar findings in all three isotopic assays of cytotoxicity, ^{51}Cr-, ^{3}H-proline and ^{125}I-deoxyuridine, and in the visual microcytotoxicity assay. It became apparent that in the past and in many laboratories, improper experimental design and inadequate statistical analysis of the data have represented serious problems, and several contributions from this laboratory were written specifically on this subject. The data in the ^{3}H-proline assay have been published. The more general conclusions we reached during these studies have also been published.

B. Model of Natural Reactivity Measured in a Short Term Cytotoxicity Assay.

By direct testing of normal peripheral blood lymphocytes against a variety of tumor cell lines, we could confirm that the myeloid line K-562 is a particularly good target yielding high and reproducible results. Other tumor cell lines tested included F-265, a lymphoid line; T-24 and RT-4, bladder carcinoma lines; MCF-7, a breast carcinoma line; G-11, presumably also a breast carcinoma line, and Chang liver cells. Levels of cytotoxicity were usually lower but equally reproducible. By competitive inhibition tests in the K-562 system using the various lines as inhibitors, it was found that solid tumor lines generally inhibited quite well, whereas various lymphoid lines, leukemic lymphoblasts, or normal peripheral blood lymphocytes would not inhibit. Thus, the competitive inhibition tests appeared quite powerful in analyzing the specificity of the natural cytotoxicity. Of interest was our finding that some lines from rats and mice inhibited quite well. More recently, we found that 7S EA complexes strongly inhibited the natural cytotoxicity. We found that Chang cell-antibody (rabbit) complexes and plastic bound TNP-anti TNP (rabbit) complexes most effectively inhibited the natural cytotoxicity. Competitive inhibition tests are now being performed in systems with target cells other than K-562 in order to extend these observations. Large

checkerboard experiments should help to resolve the question whether natural killer cells represent just one mechanism of killing and a unique specificity, or whether they show selective specificity for various binding sites or antigens. Additional testing, using peripheral blood lymphocytes from patients with various cancer, should reveal whether natural killer cells in those populations have the same properties as in normal individuals, or whether they have acquired new specificities during disease.

Inhibition studies with antigen-antibody complexes, as outlined above, strongly suggested that the effector cells in the K-562 system bear an Fc receptor, and are at least in this respect similar to the K cells mediating ADCC. We made considerable efforts to test this hypothesis. First, the role of macrophages in the K-562 system was excluded. Then, we were quite successful in removing the natural killer cells by an absorption technique on TNP-anti TNP (rabbit) coated plastic surfaces and by EA monolayers; simultaneous removal of the activity in ADCC against Chang cell antibody complexes was demonstrated. In all these experiments the absorbant seemed to have removed most of the Fc receptors positive cells as assessed with antigen antibody complexes and an indirect fluorescence technique. Recovery of these cells from the plastic surface was possible, but the natural activity was poor, as was the antibody dependent activity, probably due to remaining antigen antibody complexes on the cells' surfaces. We would not infer that the Fc receptor bearing natural killer cell is a K cell, nor that the Fc receptor plays any role in the binding to the target cell. Indeed, we have preliminary evidence to the contrary, inasmuch as various enzyme treatments had difficient effects on ADCC activity and on natural cytotoxicity. Also there is so far no evidence that an antibody is required for occurrence of natural cytotoxicity. Studies were also performed to examine previous reports that the natural cytotoxic cells might have complement receptors and could be depleted by EAC rosettes. When 19S EAC was used, only minor depletion was observed indicating that most of the natural effector cells lack complement receptors. The very strong depletion of the natural cytotoxicity by antigen antibody complexes is of utmost practical importance since it might allow to test for a remaining superimposed cytotoxicity of a different nature, namely by immune differentiated T cells. Experiments are currently performed to test whether this is indeed possible. The least we can say is that the field of natural cytotoxicity, recently so obscure and discouraging, has become one of the fascinating issues of experimental tumor immunology.

C. Cell-mediated Immunity in Patients with Cancer.

1. Cellular Immune Competence.

We further emphasized the enumeration of the percent of T cells in the peripheral blood by rosette formation with sheep erythrocytes. The test was performed under suboptimal conditions, at 29° C, as well as under optimal conditions at 4° C. The technique had been very well standardized, and this allowed considerable testing of cancer patients,

including some longitudinal follow-up studies. The earlier impression that the percentage of 29° C RFC was generally depressed in cancer patients was confirmed, and its significance was enhanced by findings such as the stronger depression in one histological type of lung cancer (anaplastic), as opposed to other types, and that the fall in the proportion of 29° C RFC occurred a few months before clinical recurrence of lung cancer. Therefore, considerable effort is now being made to systematically follow-up patients with this test and assess its value for prognosis and detection of recurrent disease. Studies are also being performed to determine the correlation between % RFC and functional activity in assays of cell-mediated immunity. Preliminary results indicate that % RFC at 29° C is a parameter representative of other commonly employed tests of general immunocompetence in our patients; however very good cell-mediated immune responses to tumor associated antigens quite often coexisted with low % RFC in the same sample of blood.

It was clearly shown during this year, in patients with lung and breast cancer, that T cell-mediated responses to the mitogens Con A and PHA, and to alloantigens in MLC, were often severely depressed. (More tests will be performed in our group of Ewing's sarcoma patients). Considerable thoughts were given to the logistics which would enable us to make such a clean discrimination from normal individuals. First, all tests were performed simultaneously with patients and with at least 3 normal responders. All responses were expressed as an index showing the relative proliferation of an individual patient's lymphocytes as compared to the mean response of the 3 normals. Thus, a relative proliferative index (RPI) was defined and calculated. Second, the percentile distribution of their own RPI around the mean response was determined for all normals in serial tests. Thus, patients with an RPI below the 10th percentile could be defined as 'low responders'. Third, a pool of cryopreserved lymphocytes from six unrelated donors was always used as stimulating cells in the MLC. Presently, we restrict the testing of general immunocompetence to Ewing's sarcoma patients, and to patients with breast and lung cancer undergoing otherwise extensive investigations on their cell-mediated responses to autologous tumor extracts. We will also test in selected patients the longitudinal course of their lymphocytes' responses to these stimuli.

2. Cell-mediated Immunity to Human Tumor Associated Antigens.

For the reasons outlined above the cytotoxicity assays and their potential use as immunodiagnostic tools have been discussed in a special section of this report, and nothing will be repeated here.

a. Leukocyte Migration Inhibition.

Further studies with the direct leukocyte migration inhibition assay have established the value of 3M KCl and more purified extracts of tissue culture lines derived from tumors, as a source of

antigen. Repeatedly 3M KCl extracts were made of the MCF-7 breast carcinoma line, which were found to specifically inhibit leukocyte migration of breast cancer patients. Soluble Sephadex G-200 and polyacrylamide gel electrophoresis purified fractions of this MCF-7 line were obtained, and some were found to induce the reaction at very low concentrations of proteins. Such highly purified preparations would then seem quite adequate for in vivo DH testing (skin testing) of patients with breast cancer, and indeed a protocol has been started to test both reactivity and specificity of skin reactions to these fractions, and to simultaneously compare reactivity in LMI to the same antigens. 3M KCl extracts of 5838, our tissue culture line from a Ewing's sarcoma also gave quite specific reactions with Ewing's sarcoma patients' leukocytes. We have been preparing 3M KCl extracts also from cultured melanoma and lung tumor cells which were used in LMI with encouraging results.

We have performed a series of experiments to examine the phenomenon reported by Black of LMI reactivity of breast cancer patients to mouse milk containing mammary tumor virus. Most patients with carcinoma of the breast have been found to react with purified mammary tumor virus prepared either from milk or from tissue culture fluids, and also with gp52, the major surface glycoprotein of the virus. The same materials elicited very little reactivity in normal women or men or in patients with other types of cancer. Studies are being actively pursued to determine the specificity and diagnostic usefulness of these findings.

A particular contribution of this laboratory in the area of LMI has been the thorough investigation of the relative contribution of mononuclear (mn) and polymorphonuclear (pmn) cells from peripheral blood in the direct capillary assay. This was first tested in a model with PPD as antigen. Highly purified mn and pmn cells were obtained by separation on a ficoll-hypaque gradient and mixtures with various percentages of each, were used in the LMI assay. It was found that the sensitivity of the assay could be significantly increased when more pmn (up to 95%) were used, than is usually the case when using unseparated peripheral blood leukocytes. For practical purposes the cell pellet after ficoll-hypaque separation was found to be a most adequate cell preparation for the LMI, having approximately this percentage of pmn; erythrocytes were lysed in part by NH_4Cl and this did not seem to affect the test. This procedure leaves most of the leukocytes from a given sample of blood at the plasma ficoll interphase available for further tests of lymphocyte functions, which would represent a definite advantage in immunomonitoring. This procedure also makes it feasible to directly test the reactivity in LMI of cryopreserved lymphocytes, separated lymphocyte fractions, lymph node cells, and lymphocytes obtained directly from tumors, by combining them with purified pmn.

Another development has been the modification of the microagarose droplet migration assay for the measurement of LMI. Initial experiments were performed with PPD as antigen, using tuberculin skin test positive and negative donors. This assay was found to be very extremely sensitive,. detecting reactivity of some skin test positive individuals at picogram

or nanogram concentrations of PPD. In addition, this assay reduces the requirement of whole blood to only 5-10 ml and permits much more extensive testing than the more cumbersome capillary tube method. This method has already proven useful in testing multiple soluble fractions from tumor cell extracts, and has detected specific reactivity to nanogram concentrations of some fractions prepared by Dr. Hollinshead and by Dr. Ralph Reisfeld.

b. Responses of Lymphocytes to Autologous Tumor Cells and Tumor Cell Extracts.

In our studies on selected patients to date, approximately 60% of our tests in lung carcinoma and 40% of our tests in breast carcinoma result in clearcut proliferation of lymphocytes to the autologous tumor material. It was found that hypotonic membrane extracts were a particularly good source of antigen in both tumor systems and these are now used exclusively in a very well standardized microtest. Patients are being studied longitudinally, and correlates are made with the other tests of immunocompetence and tumor immunity, in order to assess the clinical significance of these findings.

In addition, a macrotest in tubes is being used for the study of the production of various lymphokines in these mixed lymphocyte-tumor extract cultures. In particular, supernatants are harvested and tested in an indirect leukocyte migration inhibition assay for the presence of leukocyte inhibitory factor. Preliminary results indicate that leukocyte inhibitory factor is produced in these cultures, and that it might be a more sensitive index of response than measurement of cellular proliferation.

Cells recovered in the macrocultures in the breast system are currently being tested for the generation of cytotoxicity against the MCF-7 line. We have clearly established that a spontaneous cytotoxicity by responding cells cultured alone is present, which in all respects resemble the natural cytotoxicity. Further work is needed in this area. On top of that spontaneous cytotoxicity, lymphocytes from some patients, when stimulated with autologous hypotonic membrane extract of their tumor, develop increased cytotoxicity during the culture period. Studies are in progress to extend these observations and to characterize the effector cells.

c. Correlation of In Vivo Assays of Immunity with Clinical Status of Patients.

Over the past year we continued to utilize assays mentioned above in monitoring patients with neoplastic disease. This had included patients with carcinoma of the lung, Ewing's sarcoma, carcinoma of the breast, melanoma and carcinoma of the colon. In the first three systems this monitoring has been done in patients who are being treated under immunotherapeutic protocols in collaboration with other institutions. In the first two systems the data gathered so far were summarized and sent

for publication. Enough correlative evidence was obtained to draw some conclusions as to the value of the various tests of general immunocompetence or of tumor immunity, as outlined in various places in this report. The precise significance of each set of data and tests for the prognosis of each individual patient remained to be defined however. Thus, a considerable effort was made to set up the logistics which would allow a most regular, longitudinal, follow-up of each patient with what seemed the most important tests, as mentioned at several places in this report. It was found that the logistics were improved and would allow the proper conclusions to be made on these studies.

d. Search for Immunosuppressive Factors and Contaminants of Tumor Cells.

Over the past two years we have developed in vitro techniques which allowed us to recognize immunosuppressive characteristics of tumor cells which affect the generation of cytotoxic lymphoblasts. We then found in a mouse tumor system, and in several rat tumor systems, that parvoviruses which contaminated those tumors were responsible for the observed inhibition. Highly purified Kilham rat virus was able to suppress various immune responses in vitro and in vivo in the rat and this was associated with a cytopathic effect on the responding lymphocytes. In the mouse the purified virus (minute virus of mice) was tested only in vitro, and was found to affect selectively the generation of cytotoxic blasts, without detectable cytopathic effects on other cells. During the course of these studies, similar effects with mycoplasma organisms were described by other groups. These results are of paramount importance for the understanding and the prospective design of all in vitro experiments of tumor immunology using these systems. Furthermore, it is now clear that the rat parvovirus has some enhancing effects in vivo on tumor growth and a role of the virus for the original establishment of these tumors must be seriously considered.

We have undertaken a similar study in man, screening for the immunosuppressive characteristics of tissue culture lines derived from tumors, and of fresh tumors. Preliminary results indicate that some of the tissue culture lines are inhibitory, and these are now under investigation for possible contaminations. So far, fresh lung carcinoms cells have not been found inhibitory, except in very few instances. Clearly, these areas need further testing.

IV. Experimental Models in Animals.

During the fiscal year 1974, the earlier principal investigator on this project and his associates had accumulated considerable experience of various assays of cellular immunity in animal tumor models. Progressively, during this FY, the animal studies were phased out or transferred to another project, thus allowing more work to be done in human tumor systems. Considerable efforts were made to summarize the animal

data for publication, as will appear here and in the list of publications in the FY 1977 report. Since these data have been adequately summarized in the previous annual report, they are only mentioned again here in the "Summary of Work". Selective areas, which are still under intensive investigation have been transferred to Project No. Z01 CBD 05557-08 LID and will be delt with on that annual report. Most of the animal work of the new principal investigator on this present project will also be delt with in the report Z01 CBD 05557-08 LID.

V. Significance of Biomedical Research and the Program of the Institute.

The use of various forms of immunotherapy in man has elicited considerable interest. It is imperative that the efficacy of such treatments be evaluated by clinical follow-up, and that the mode of action on the immunological system, if any, and the role of the immunological system during such therapy be clarified. This would fall under Objective V, Approach 4 and Objective VI, Approach 1,2 and 4 of the National Cancer Plan.

The available techniques for the solubilization and isolation of tumor associated cell surface antigens are important in their application to immunological monitoring, as described in this report. Such antigens may perhaps also be useful for therapy in the future, and the use of such purified antigen preparations for immunotherapy would fall under Objective VI, Approach 1,2 and 4 of the National Cancer Plan.

The ability of our laboratory to fractionate subpopulations of cells responsible for natural activity and cell-mediated immunity to tumor antigen should allow detailed dissection of the role of the immune response in eliciting or preventing tumor growth. These studies may also be useful in the immunodiagnosis of cancer and in measuring changes of immunity in response to chemotherapy or immunotherapy. This would fall under Objective V, Approach 4 and Objective VI, Approach 1 and 4 of the National Cancer Plan.

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. The development and standardization of <u>in vitro</u> assays for cell-mediated immunity is one of the most important aspects of tumor immunology. Because immunogenicity cannot be studied directly in the human, the evaluation of immunologic reactivity and the antigenicity of tumor cells by these assays is of paramount importance. This would fall under Objective V, Approach 4 of the National Cancer Plan.

The question of whether immunosuppressive factors are associated with cancer cells is also very important. It appears that the stimulation of the host immune system may be useless, unless one can first rid the body of the inhibitory phenomena related to tumor cell growth. Investigations in this area would fall under Objective IV, Approach 4 and Objective VI, Approach 1,2 and 4 of the National Cancer Plan.

VI. Proposed Course of Project.

This project represents our laboratory's commitment to understanding the role of the host immunological response to the tumor and tumor progression. As has been emphasized previously in this report, the functional integrity of each cell type in the immune system, as demonstrated by intact cell-mediated and humoral natural and immune reactions, as well as the capacity of particular cell types to mount their aggressive response to the tumor, may be of paramount importance and should represent one of the ultimate goals of our research.

Our studies of the cell-mediated reactions, relative to general immunocompetence, natural cellular reactivity, and specific cellular immunity in human tumor systems will be continued. The relationship between the results achieved in various assays, and the clinical course of patients undergoing surgery, radiation therapy, or immunotherapy will be examined. The potential of each of these assays for the immunodiagnosis and/or immunological monitoring of cancer will be carefully examined. The effect of different immunotherapeutic modalities, such as BCG, Corynebacterium parvum, and tumor cell extracts will be further examined through continuation of our randomized clinical trials and the use of our immunological assays to monitor these patients.

During the coming year, some emphasis will be put on thorough longitudinal studies on lung cancer patients with surgically resectable disease randomized to no therapy, chemotherapy alone, or chemotherapy plus BCG and allogeneic cells. The laboratory will organize itself to follow-up such patients autonomously at the Clinical Center, NIH. A protocol with high dose methotrexate and citrovorum factor rescue with or without BCG is foreseen for patients with more advanced disease at the collaborating institutions. Collaborative research with Litton-Bionetics, Inc. and George Washington University will allow us to grow tissue culture lines derived from tumor of the appropriate histological type, and to prepare soluble purified fractions, as described. Then, in all tumor systems these will be tested simultaneously for eliciting delayed hypersensitivity reactions and leukocyte migration inhibition. This in vivo/in vitro correlate should be useful to characterize better the fractions which might indeed contain tumor associated antigens. It is hoped that some antigens will show tumor and tissue type specificity. Tests of proliferative and cytotoxic responses will also be performed, mainly with crude extracts of autologous tumors as antigen; additionally the production of leukocyte migration

inhibition factor in these cultures will be assessed in parallel. Later on the responding cells in these assays will be studied according to their origin in different lymphoid organs, or in the tumor itself. Lymphoid cells will be characterized and separated for use in the assays, according to their cell surface characteristics. The role of the cell hitherto known as the K cell will be assessed and compared with the characteristics of the cells mediating natural reactivity. Cell separations will be monitored by morphological and functional studies independent of the fractionation technique, and whenever possible two or more ways of obtaining a given subpopulation of cells will be used. Particular attention will be devoted to the kinetics, the dose-response curves, and the mechanisms of the reactions. Insight into the latter will be obtained by the use of metabolic inhibitors with well described activity in other types of cellular immune reactions. Thus, in addition to our continued commitment to the follow-up of cancer patients, it will be possible to gain essential insights into the mechanisms of the host response to the tumor, much along the lines of many animal studies in this laboratory but with definite advantage of studying the species for which cancer research is ultimately directed.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08511-01 LID

PERIOD COVERED

August 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization and Measurement of Soluble Antigens Associated with Neoplastic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.R. McIntire	Senior Investigator	LID NCI
OTHERS:	J. Braatz	Staff Fellow	LID NCI
	A.-K. Ng	Visiting Fellow	LID NCI
	H.T. Holden	Microbiologist	LID NCI
	G.D. Bonnard	Visiting Scientist	LID NCI
	J. Weese	Guest Worker	LID NCI
	G.L. Princler	Chemist	LID NCI

COOPERATING UNITS (if any)

Metabolism Branch, NCI; Laboratory of Chemical Pharmacology, NCI; Surgery Branch, NCI; Epidemiology Branch, NCI; National Naval Medical Center, Bethesda, Md.; and AFIP, Walter Reed Army Medical Center, Washington, D.C.

LAB/BRANCH

Laboratory of Immunodiagnosis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.00	3.00	1.00

SUMMARY OF WORK (200 words or less - underline keywords)

Circulating tumor Markers are being studied to aid in the detection and diagnosis and monitoring the course of patients with various neoplastic disease. Sensitive quantitation of alpha-fetoprotein (AFP) by radio-immunoassay has been shown to be of considerable value for following therapy in patients with hepatocellular carcinoma (HC) and testicular cancer of germ cell origin where quantitation of human chorionic gonadotropin (HCG) also serves as an indicator of tumor activity. The usefulness of AFP in non-malignant disease such as congenital neural tube defects and infectious hepatitis is also being studied. Experimental studies in primates with chemically-induced HC allow the demonstration of the usefulness of AFP for early detection of tumor and also to calculate the synthetic (rate) and catabolic rate of AFP to derive the total body load of tumor cells. Progress continues on the isolation of a lung tumor associated antigen (LTAA) using physicochemical and immunochemical techniques. Isolation will be monitored by radial immunodiffusion, leukocyte migration inhibition (LMI) and by delayed hypersensitivity skin testing. After antigen purification a RIA will be developed for the assay of patient serum. Similar studies are being performed using a murine virus-induced leukemia (MBL-2) as a model system and inhibition of ^{51}Cr release cytotoxicity by antibody and complement as the antigen detecting system.

Project Description:

This project is a continuation of work previously done under CBD 05554 under the Laboratory of Cell Biology.

I. Characterization and measurement of soluble antigens associated with human neoplasms. Objective 6, Approach 1

A. Studies of Alpha-Fetoprotein (AFP)

1. Quantitation of AFP in human disease: We are continuing to perform assays for AFP to provide evidence that this marker protein is of great value for following therapy in certain types of neoplastic disease. We are collaborating with various investigators in the Washington area and in other parts of the United States to obtain serial serum samples from patients with hepatocellular carcinoma, embryonal carcinoma of the testis and pancreatic carcinoma. We have been able to demonstrate a dramatic response to adriamycin in at least one hepatoma patient where the serum AFP measure proved superior to clinical measures for extent of disease (manuscript in preparation). AFP together with human chorionic gonadotropin (HCG) has proven to be an excellent marker for following the response to chemotherapy in testicular tumors. An important aspect in this disease is the ability to make an early diagnosis of recurrent tumor by noting a rise in either or both markers. There is preliminary evidence to indicate that using these markers will aid significantly in detecting those patients with retroperitoneal node involvement or other metastatic disease folowing primary orchiectomy.

A study of a large number of amniotic fluid samples (237) has provided the best "normal curve" for AFP yet to be published and emphasizes the need for a sensitive radioimmunoassay (RIA) if one is to diagnose neural tube defects (NTD). In all 33 patients with confirmed NTD the amniotic fluid AFP was above the mean + 2 SD for that age of gestation. The AFP in NTD was considerably higher in the 1st 26 weeks of gestation and came closer to the normal range in the last trimester.

Further studies of serum AFP levels in patients with non-neoplastic liver disease indicates that the diseases with greatest elevations of AFP are those associated with hepatitis virus. The elevation of AFP characteristically occurs after that of the liver enzymes and may be a marker for liver regeneration.

2. Study of AFP in primates: Study of serial serum AFP levels in primates undergoing hepatic carcinogenesis have provided much basic information. Monkeys started on diethylmitrosamine (DENA) at birth develop a rising AFP and hepatoma at an earlier time than monkeys started on DENA at 12 months of age; the difference is much greater than the 12 months delay in starting the carcinogen. Is is also quite clear that the Rhesus species develops liver cancer sooner than the Cynamolgus and that for both species the male is affected sooner than the female. These

differences might not have been noticed and would not be easily documented without a quantitative measure of AFP (manuscript in preparation). We are now entertaining a retrospective study to look for differences in the histopathology of hepatomas producing large amounts of AFP and those producing smaller amounts.

3. Metabolism of AFP: Studies on in vitro synthesis by freshly obtained biopsy specimens of hepatoma are continuing. We have determined the proper technical procedure to insure a linear synthesis of AFP for 24 - 72 hours which provides some assurance that the in vitro conditions approximate the in vivo. Synthetic rates of various hepatomas in the short term culture experiments and some using an established tissue culture line indicate that the capacity of the average cell is between 0.008 and 0.024 pgm/day. Tumors coming from monkeys with higher levels of AFP appear to have a greater synthetic rate, but work remains to be done before we can correlate AFP with tumor cell number.

The turnover of AFP was determined in 15 normal adult Rhesus monkeys using native AFP and the half-life was 3.5 - 4.0 days with a fractional catabolic rate from 25 - 35% of the i.v. pool per day. Radiolabelling of AFP with ^{125}I using 3 different methods (iodine monochloride, electrolytic and lactoperoxidase techniques) all produced significant biological denaturation of the AFP (30 - 60%) which could be removed by zone electrophoresis in cellulose acetate. The labelled AFP then was handled identically to native AFP. Studies on turnover of AFP in hepatoma-bearing monkeys are now in progress.

4. Use of AFP in combination with other markers: As mentioned above, AFP and HCG have proven to be excellent markers to indicate the activity of testicular tumors.

Both gastric and pancreatic carcinoma have been noted in the past to be associated with CEA, AFP and HCG. We are now engaged in a retrospective study with Dr. V.L.W. Go, (Mayo Clinic) to determine the value of simultaneous measurement of all three markers. At the present time 47 patients with pancreatic cancer have been evaluated and the use of combined markers demonstrates a clear superiority. Twelve patients had only one marker while 6 more had two markers elevated; no patients had elevations of all three. Utilizing this type of information we have analyzed serums from patients in the Framingham Heart Study who developed gastric or pancreatic cancer. The results showed that CEA was the most frequently elevated but this occurred in control patients as well; addition of a second elevated marker was only found in two patients with cancer (manuscript in preparation).

5. Another use of combined markers has been to use specific antiserum for staining testicular tumor tissue for AFP and HCG using a horseradish peroxidase label. Preliminary results indicate a high level of correlation with elevation of the marker in the serum. Since this technique can be used on formalin-fixed tissues it may become an aid in the initial

diagnosis of testicular tumors and one that could provide information regarding effective chemotherapeutic agents.

B. Studies of Lung Tumor Associated Antigens: Objective - 5, Approach - 4

1. The isolation of a human lung tumor associated antigen (LTAA) has proceded along several fronts. Utilizing techniques devised in previous years, we have used malignant pleural effusions as starting material. The use of DEAE gradient chromatography after the fluid had been treated with pH4.5 dialysis to precipitate unwanted protein and then 50% ammonium sulfate to further remove unwanted protein was helpful. The antigen containing fraction from DEAE cellulose gradient elution still contains several bands on analytical acrylamide gel electrophoresis, but represents an easy procedure for this relative purity using very mild treatment steps.

In addition we have used antibody coupled to Sepharose to prepare an affinity column to isolate the LTAA by direct absorption. The antiserum (R-152) was raised against a pooled PCA extract of several lung tumors and absorbed with extracts of normal lung and other tissue so that it gives a reaction with lung tumor extracts but not with equivalent extracts of normal lung. A saline extract of metastatic lung tumor (to liver) gives two lines of reactivity with R-152 and after processing through the affinity column still retained 2 lines of reactivity by double diffusion in agar gel. Acrylamide gel electrophoresis of this preparation showed 4 major protein components. Further purification by ion exchange chromatography yielded a preparation which retained both lines of immunological activity and had two major bands on acrylamide gel electrophoresis. Further steps will attempt to isolate the single antigen components for development of a RIA. Normal lung extract is being processed in an identical fashion and both quantitative and qualitative differences from the tumor are noted. Development of "second generation" antisera, sensitive quantitative assays such as RIA, and screening appropriate clinical serum samples are planned for the coming year.

2. Attempts to quantitate the LTAA by hemagglutination proved to be non-specific so that diffusion in antibody containing gel has now been tried. Although less sensitive than hemagglutination and RIA, single diffusion in agar does not require a highly purified antigen. The preliminary studies indicate that a radial diffusion method is more sensitive than electro-immunodiffusion.

3. Efforts to detect HLTAA in fixed tissues are continuing. Problems of specificity and technical problems need to be resolved, but this technique could provide a diagnostic advantage for cytologic examination of sputum or bronchial washings.

4. We have developed a leukocyte migration inhibition (LMI) to test for cell mediated immunity in patients with lung cancer and will use

it to assess the antigen isolation proceeding by monitoring with xenogeneic antisera. An effort is now underway to isolate human leukocyte inhibition factor (LIF) by affinity chromatography. A RIA against this lymphokine could be used to potentiate or replace the LMI assay and could yield valuable information concerning the mode of action of LIF.

II. Tumor Associated Antigens in Mice: Objective - 3, Approach - 5

A. Isolation of surface antigen(s) from a Moloney leukemia virus induced tumor (MBL-2)

1. Development of in vitro assay methods for quantitation of the antigen.

a. Humoral immunity assay - Inhibition of ^{51}chromium release cytotoxicity assay. Cytotoxic antibodies against MBL-2 cells were produced in the syngeneic system by immunizing C57BL/6 mice with murine sarcoma virus (MSV) and in the xenogenic system by immunizing rabbits with MBL-2 cells. A microcytotoxicity test was developed which could be used to detect antigen(s) either on whole cells or as soluble cell extracts.

b. Cell mediated immunity assay - Macrophage migration inhibition assay. Spleen cells harvested from C57BL/6 mice 10 days after inoculation with MSV (regressor) can be specifically stimulated by MBL-2 cells to produce macrophage migration inhibition factor. This factor can be measured by the inhibition of macrophage migration from a capillary tube or from an agarose gel drop.

2. Isolation of antigens from MBL-2

a. MBL-2 cells have been treated with 3M KCl to extract surface antigens. The crude extract was found to contain antigens as measured by both the inhibition of cytotoxicity and the inhibition of migration assays.

b. The 3M KCl extract was fractionated by ion exchange and gel filtration chromatography. Preliminary assay of the fractions indicated the possibility of more than one antigenic species.

B. Immunoglobulin (Ig) markers on the surface of plasma cell tumors in mice.

Previous studies suggested that BALB/c mice, which have been injected with mineral oil intraperitoneally to stimulate the development of plasmacytomas, generated cytotoxic antibody which became adherent to plasmacytoma cells which could then be lysed by the addition of rabbit or guinea pig complement. Further testing indicates that approximately 18% (11/60) of original plasmacytoma serums tested were able to cause > 20% net lysis of tumor cells in the presence of complement.

A time course study on the acquisition of the putative host anti-tumor antibody indicates that as early as 8 days after inoculation of tumor 4-33% of the tumor cells can be lysed by the addition of complement only. The percentage of complement succeptible cells does not change markedly with time in the host animal, but may increase to levels of 50-60% or even greater net lysis in some animals four to five weeks after inoculation.

III. Significance for biomedical research

There are, at the present time, many circulating substances which can be assayed in the blood of individuals with neoplastic diseases which are reported to be associated with the tumor process. Some of these substances are obviously more specific for malignancy than others. Those substances that are the most specific have the potential for being utilized as aids in early diagnosis (possibly for screening) and for localization of tumor, but have the immediate advantage in serving as "tumor markers" to follow the effectiveness of therapy. All internally located solid tumors have shared a great disadvantage in not being easily measured or otherwise evaluated for progression or regression. This laboratory is committed to the demonstration of clinical application of known "tumor markers" (AFP and HCG) and to the discovery of new ones (LTAA). In addition to demonstrating an association between certain tumors and markers, we are interested in studying the metabolism of the markers to further define the precise relationship. It is possible that the markers or immunological reagents directed against them could have some value in immunotherapy or even in prevention. This research on tumor markers falls under Objective VI, Approaches 1 and 2 and Objective V, Approaches 2,3 and 4 of the National Cancer Plan.

The study of cell membrane antigens and ways to detect and quantitate them will provide basic information needed to understand the process of carcinogenesis and malignant transformation. Animal model systems and in vitro systems are frequently used because of the ease of manipulation and control of variables. We are searching for membrane-bound antigens since those on the surface of the cell are the most likely to trigger immune recognition and also serve as possible targets for immunologically directed therapy. This research falls under Objective III, Approaches 4 and 5.

IV. Proposed course of project

This project represents our laboratory's continued commitment to immunodiagnosis utilizing both circulating antigens, released or secreted by tumor cells, and cell surface antigens. We will continue to provide information as to their usefulness in following the course of disease and how they may be used in combination to aid the diagnosis of cancer.

We plan to continue using the RIA for AFP and HCG to monitor patients with hepatoma, testicular cancer and pancreatic cancer. Addition-

ally we will utilize the carcinogen-induced hepatomas in primates to study the relationship of AFP to tumor burden by measuring synthetic rates and catabolic rates of AFP in the tumor condition. We plan to continue studying AFP in non-malignant liver disease as an example of dealing with the aspect of lack of specificity found with all current tumor markers. We will stop investigating maternal and amniotic fluid AFP levels associated with neural tube defects. We will continue to do tissue staining for HCG and AFP as an aid to histological classification in testicular tumors.

We hope that in the coming year we will be able to start using a RIA for a lung tumor associated antigen and demonstrate a clinical usefulness in diagnosis or following the course of primary lung cancer or both.

We will continue to search for tumor associated antigens in mouse tumors to extend our basic knowledge of the process of transformation and of the host response to tumor. The techniques applied in the animal model system are transferable with minor modification to the investigation of human malignancy. As this work grows we will phase out the studies on immunoglobulin surface markers on plasma cell tumor cells.

Publications:

Sizaret, P., Breslow, N., Anderson, S.G., Adinolfi, M.C., Buffe, D., Goussev, A., Kohn, J., Krebs, B., Lehmann, F.G., Martel, N., McIntire, K.R., Nishi, S., Purves, L., Rowe, D.S. and Ruoslahti, E.: Collaborative study of a preparation of human cord serum for its use as a reference in the assay of alpha-fetoprotein. J. Biol. Stand. 3: 201-223, 1975.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08517-01 LID

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular and Tumor Immunity in Experimental Animal Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.T. Holden	Senior Investigator	LID NCI
OTHERS:	R.B. Herberman	Chief, LID	LID NCI
	S. Landolfo	Visiting Fellow	LID NCI
	L.W. Redmon	Biologist	LID NCI

COOPERATING UNITS (if any)

Litton Bionetics, Inc. Viral Biology Branch, NCI
Medical University of South Carolina
Laboratory of Experimental Chemotherapy, NCI

LAB/BRANCH

Laboratory of Immunodiagnosis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 2/3	1 5/6	5/6

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year the major focus of this project has been to study the mechanisms involved in the immune response to tumor antigens in experimental animal systems. Our approach has been multifaceted, employing numerous in vitro assays to gain insight into the immune reactions, and performing diverse in vivo experiments to determine which immune functions may play a role in tumor growth or regression.

T cell-killing of tumor cells by lymphocytes has been studied in cytotoxicity assays (^{51}Cr and ^{125}IUdR release) using immune cells isolated from the tumor. Additional testing on the in vivo secondary response confirmed the in vivo role of the cytotoxic immune function. Studies on the specificity of the cytotoxic response have continued with emphasis on the possible role of H-2 antigens in syngeneic T-cell killing. Another T-cell dependent function, production of migration inhibition factor has been extensively studied in order to expand our understanding of the tumor immune response. We have continued investigations into the role of the suppressor and growth inhibitory cells on the cellular and tumor immune response. Finally, we have continued work in the area of cryopreservation of immune function using normal and immune lymphoid populations and tumor cells for in vitro assays and for in vivo studies.

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and to detect quantitative changes in normal antigens on malignant cells; (2) to detect cellular immune reactions to tumor antigens; (3) to develop and standardize in vitro assays to measure cell mediated immunity to tumor antigens; (4) to correlate the findings of the in vitro assays with the growth of the tumor in the host; (5) to evaluate the diagnostic, prognostic and therapeutic significance of cellular immune responses to tumor antigens and to evaluate the effect of tumor substances (such as antigen, antibody and antigen-antibody complexes) on these cellular immune responses; (6) to measure general immunological competence of tumor bearing animals, and to determine the relationship between this and the growth of the tumor.

Methods Employed: Host lymphoid cells are obtained from the spleen, lymph node, peritoneal cavity, peripheral blood, thymus and/or tumor of tumor-bearing, immune and control animals. A variety of normal and tumor cell lines are maintained in tissue culture. A variety of tumors induced by viral, chemical or unknown agents are maintained by transplantation in syngeneic animals. Immune and normal lymphocytes and tumor cells are stored in a viable frozen state. Inbred strains of mice are employed for several different allogeneic and tumor model systems. Immunological competence and/or cellular immune reactivity are measured in vitro by the following tests: lymphocyte stimulation by mitogens, lymphocyte-mediated cytotoxicity (^{51}Cr and ^{125}IUdR release), inhibition of lymphocyte-mediated cytotoxicity, production of migration inhibition factor, generation of primary and secondary cytotoxic responses, suppression of mitogen-induced proliferation, and inhibition of growth of lymphoma cells. In vivo, immunity was assessed by adoptive transfer and by challenge experiments. Animal tumor cell membrane and soluble fractions were prepared for antigenic study. Lymphoid populations were enriched or depleted of different subpopulations by iron-magnet treatment, passage over adherence columns, treatment with antisera plus complement, or adsorption on antigen or antigen-antibody monolayers.

Major Findings:

I. Tumor Immunity in the MSV Tumor System.

Murine sarcoma virus (MSV) when injected into normal C57BL/6 mice produces tumors which appear 7-9 days after injection, reach peak size at 12-13 days, and regress by 21-25 days. Peak cytotoxic reactivity, as measured by a 4 hour ^{51}Cr release, a 48 hour ^{125}IUdR release or visual cytotoxicity assay, occurs at about 14 days after injection.

A. Specificity of the cytotoxic reactivity.

Previously it has been demonstrated that cytotoxic reactivity, as measured by the 4 hour ^{51}Cr release cytotoxicity assay, of spleen cells from C57BL/6 mice injected with MSV 14 days earlier was T cell-dependent

and was directed against endogenous type C virus antigen, designated MEV-SA-1. At first there appeared to be an association between the H-2 antigen and the MEV-SA-1 since it was mostly syngeneic targets that were lysed by the effector cells. However, if the allogeneic target cells were grown in vitro, this antigen could be detected by the 4 hour inhibition of lymphocyte-mediated ^{51}Cr release cytotoxicity assay. In addition, different endogenous type C viruses isolated from these tumor lines could cause expression of MEV-SA-1 on feral mouse cells and also on xenogeneic rabbit cells as detected by the inhibition assay. Since most of the cross-reacting antigens on allogeneic targets had been detected by use of the inhibition assay, and since several other groups have stressed the strong association of H-2 antigens with the specificity of cytotoxic T cells, we started to employ a more sensitive 18 hour ^{51}Cr release assay to examine the interrelationship between the H-2 antigen and the T cell-dependent cytotoxic reactivity. Most of the allogeneic targets which were positive in the inhibition assay after they had been cultured in vitro were also positive in the direct 18 hour test, although the levels of cytotoxic reactivity were generally not as high as against the syngeneic targets. Treatment with anti-Θ plus complement indicated that the cytotoxicity against tumor antigens on the allogeneic targets was also T cell-dependent.

B. Kinetic pattern of tumor growth and cytotoxicity.

Injection of different concentrations of MSV could influence the pattern of tumor growth and the kinetics of cytotoxicity. Using a single virus stock in which > 90% of the animals developed tumors with virus dilutions between 1/10 and 1/80, significant differences in both parameters were observed. With the lower dilution of virus (1/10) maximun tumor size occurred earlier (around 11 days after injection) than with the high dilution (16-17 days). Cytotoxic reactivity was also a function of the amount of virus used for injection, with lower dilutions of virus inducing peak reactivity sooner than higher dilutions. Nevertheless, the relationship between peak tumor size and maximum cytotoxic reactivity remained the same with the latter occuring immediately after the tumor started to regress.

Studies of this nature emphasize the problems which may occur when comparing investigations on tumor growth and in vitro testing performed by different laboratories. Discordant data may be the result of different amounts of virus (or different virus stocks). But, as noted, within each virus concentration the relationship between the in vitro tests and tumor growth was similar.

C. In vivo secondary cytotoxic response.

We have previously demonstrated that animals immunized with MSV undergo a secondary cytotoxic response when challenged with tumor cells carrying the appropriate antigens. Further studies have shown that the ability to detect cytotoxic reactivity was a function of the length of time between primary and secondary challenge, the site at which the animals were

challenged, the site from which effector cells were harvested, the length of time between the secondary challenge and the harvest of the effector cells, and the size of the challenge inoculum. Each of these variables acted independently to influence the kinetics of the secondary cytotoxic response, making it difficult a priori to determine the optimum time for detecting a secondary cytotoxic response. Hence, if MSV immune animals were challenged intraperitoneally or subcutaneously within one month after primary immunization, cytotoxic reactivity could be detected in many lymphoid organs for varying lengths of time. However, if the time between primary and secondary injections was greater than 3 months, cytotoxic reactivity tended to be localized at the site of challenge (especially when the animal was challenged subcutaneously) and could be detected only for a very short period of time.

D. In vivo activity of growth inhibitory and suppressor macrophages.

Macrophage mediated growth inhibitory activity was observed in vitro with spleen cells from animals injected with MSV or Corynebacterium parvum (C.p.) 14 days earlier. If these spleen cells were mixed with lymphoma cells and incubated for 48 hours in vitro, there was a pronounced inhibition of the proliferation of these tumor cells. Tumor cells which did not carry detectable cross-reacting antigens were inhibited to a similar extent indicating that this was a nonspecific effect. In addition, macrophages from these animals could suppress the response to PHA as well as the in vitro generation of primary and secondary cytotoxic responses.

Experiments were performed to determine whether the growth inhibitory and suppressor activities of macrophages had significant effects in vivo or whether they could only be detected as in vitro phenomena. When MSV tumor bearing animals were injected with the allogeneic P-815 mastocytoma, the primary cytotoxic response to this tumor was often decreased when compared to normal controls, presumably due to the suppressor cells which were induced by the tumor. In addition, if MSV immune animals were challenged with X-irradiated RBL-5 tumor cells mixed with suppressor spleen cells from animals injected with C.p. there was an inhibition of the secondary cytotoxic response. Similarily, if animals were injected with C.p. and then injected with MSV, the level of cytotoxic reactivity was reduced at day 14 when compared to controls. Macrophage mediated growth inhibitory activity could also be detected in vivo. Spleen cells from animals inoculated 14 days earlier with MSV were treated with anti-Θ plus complement to eliminate the cytotoxic T cells. These cell suspensions, when mixed with RBL-5 lymphoma cells and inoculated subcutaneously into normal, syngeneic mice, inhibited the tumor growth whereas normal spleen cells did not.

Hence, both in vitro and in vivo, macrophages from tumor bearing or C.p. treated animals can have both immunosuppressive and antitumor effects. It remains to be determined how these functions influence tumor growth in various in vivo situations.

E. Cytotoxic, growth inhibitory and suppressor cells isolated from MSV tumors.

A number of different immunological responses are evoked in animals after a primary challenge with murine sarcoma virus. These responses are both specific and nonspecific, cell-mediated and humoral. Almost all studies on cell-mediated immunity in this and other systems have been performed with cells from peripheral lymphoid organs. On the assumption that the most important immunological responses against the tumor should be occurring at the site of tumor growth, we have initiated a study to evaluate the ability of host cells within primary MSV-induced tumors to function as effector cells in vitro. Host cells were obtained by enzymatic disaggregation of the tumor and fractionated by sedimentation velocity at unit gravity on a Ficoll gradient. Characterization of these cells indicated that 30-40% were T lymphocytes, about 50% were macrophages and less than 5% were B lymphocytes. Two different functionally active populations have been isolated to date. T lymphocytes have been identified in the tumors by their surface markers as detected by indirect immunofluoresence using rabbit anti-mouse brain antiserum and by their functional reactivity as measured by a 4 hour ^{51}Cr release cytotoxicity assay. In addition, macrophages were also found within the MSV tumors as determined by phagocytosis, the presence of an Fc receptor on their surface and functionally by their ability to inhibit the proliferation of lymphoma cells in a growth inhibition assay (GIA) and by their capacity to suppress the PHA induced proliferation of normal spleen cells. When the cells from the tumor were fractionated, the T cells had a sedimentation velocity of 3.5-4.0 mm/hr while the macrophages subdivided into two populations, one which sedimented at 4.0 mm/hr and another at 6.0 mm/hr. Using phagocytosis of antibody coated sheep erythrocytes (EA) it was possible to distinguish the two subpopulations of macrophages. Those cells sedimenting at 4.0 mm/hr would readily opsonize EA while macrophages sedimenting at 6.0 mm/hr did not, although they could bind EA through the Fc receptor. Evidence obtained to date would indicate that the cell which mediates growth inhibition is the same cell that is capable of suppressing the PHA stimulation of normal mouse spleen cells. The anti-tumor effect of the T cells was antigen specific in contrast to the activity in the GIA which was directed against cells which did not carry detectable cross reacting antigens.

F. Adoptive transfer experiments.

Previously we have performed adoptive transfer experiments which demonstrated that immune spleen cells could transfer immunity against MSV challenge in X-irradiated recipients and that this protection was T cell dependent. These studies were continued in two areas. First, using cryopreserved spleen cells we were able to protect X-irradiated mice against challenge with MSV, indicating the feasibility of using this source of cells for repeated prophylaxis or immunotherapy studies. Second, adoptive transfer experiments were performed in which intact tumor cells were employed for challenge, instead of tumor viruses, to eliminate the possibility that virus neutralizing antibody was mediating the protection which we were

seeing. Immune spleeen cells were capable of transferring resistance to challenge with Rauscher lymphoma, RBL-5 or with an MSV-induced tumor, MSB.

G. Cell-mediated immunity to MSV as measured by MIF production.

Extensive studies have been performed using the migration inhibition assays in order to determine the relationship of this assay to the growth and regression of MSV tumors. Both the direct capillary and the indirect agarose assays have been utilized to assay the production of migration inhibition factor (MIF). Using RBL-5 cells as a source of antigen, the kinetics of the response in MSV injected animals as seen in the migration inhibition assay were quite different from those described for the 4 hour ^{51}Cr release cytotoxicity assay. At day 9 after MSV injection, there was a sharp increase in activity which peaked at day 10 and then rapidly declined to very low levels by day 14, the peak of cytotoxic reactivity. A second peak of MIF production was observed at 17-19 days after inoculation and by day 21 the reactivity had subsided. Individual animals that were tested in parallel for MIF production and for cytotoxic activity in the 4 hour ^{51}Cr release cytotoxicity test had strong reactivity in one assay and low activity in the other assay and vice versa. Activity in both assays was T cell-dependent since it could be removed by treatment with anti-θ plus complement.

Different tumor lines were employed as a source of antigen to determine the specificity of the reactivity. Broadest specificity was seen 9 days after inoculation at which time the immune cells produced MIF in response to syngeneic and allogeneic cells containing MEV-SA-1 and/or Moloney leukemia virus-associated antigens. At day 14, only a syngeneic tumor (MBL-2) containing MEV-SA-1 and Moloney antigen gave consistently positive results. At 17 days both RBL-5 and MBL-2 were able to stimulate MIF production.

The kinetics of reactivity were different when a 3M KCI soluble extract of RBL-5 was used as an antigen source. MIF production was low but significant at 9 days after injection with MSV, rose to peak production at 14 days and then declined by day 21.

Hence, lymphocytes from animals immunized with MSV are capable of producing MIF in responce to antigenic stimulation. Depending on the source and the specificity of the antigen, the kinetics of this reactivity may vary with activity detectable between 9 and 21 days after injection.

II. Cryopreservation of Normal and Immune Lymphocytes.

Extensive studies have been performed in both mouse and rat experimental systems to determine the optimum conditions which must be employed to cryopreserve viability and functional reactivity of normal and immune leukocytes and of tumor cells. We have determined that DMSO concentrations of 7.5-10.0% and a freezing rate of -1° to -2°C/minute are necessary to achieve the best recovery of cell activity. In addition, after the

cells have been thawed, the DMSO must be diluted out slowly, dilution should be accomplished at room temperature, and the cells should not be subjected to mechanical or physiological stress. Furthermore, if the cells are to be held for any length of time after recovery, this should be done at room temperature, not 0°C.

Using these techniques it has been possible to reliably freeze lymphocytes and tumor cells. In fact, using frozen effector and frozen target cells as an internal standard, we have been able to standardize the ^{51}Cr release cytotoxicity assay to enable direct comparison of results obtained on different days of testing. In the mouse system, we have been able to cryopreserve normal lymphocytes so that they are able to respond to mitogen stimulation after thawing, and to preserve macrophage activity as measured by the suppressor cell assay. Within the rat system, we have cryopreserved cytotoxic functions (4 hour ^{51}Cr release and 48 hour ^{3}H-proline and $^{125}IUdR$ release), mitogen response, mixed lymphocyte-tumor cell responses, secondary cytotoxic reactivity and growth inhibitory activity of macrophages.

Lastly, we have explored using cryopreservation to differentially remove subpopulations of T lymphocytes. Two funcionally distinct populations of T cells are more susceptible to cryopreservation: normal lymphocytes which are capable of responding to PHA and cytotoxic lymphocytes that are directed against alloantigens. Mitogen response to Con A and endotoxin, cytotoxicity against tumor antigens and normal cytotoxicity are all more resistant to freezing damage.

Significance to Biomedical Research and the Program of the Institute:

Detection of tumor antigens and of immune reactions to tumor specific and tumor associated antigens is essential for progress in tumor immunology. The sensitive assays for humoral and cell-mediated immunity which have been developed and standardized in the laboratory have already proved useful in studying the immune response to animal and human tumors. *In vivo* experiments performed in conjunction with the *in vitro* assays assist in determining which tests accurately reflect the important immunological events taking place in the host to influence tumor growth.

The ability of our laboratory to perform multiple assays of humoral and cell-mediated immunity to tumor antigens should allow detailed dissection of the role of the immune response in limiting or preventing tumor growth. These assays may also be useful in immunodiagnosis of cancer and in measuring changes in immunity, in response to chemotherapy or immunotherapy.

Proposed Course of the Project:

During the next year we will continue to study the immune responses to tumor associated antigens in experimental animals in several different areas.

Since the immune response to tumor growth is most important to the host at the site of the tumor, we will expand our investigations into this area by conducting studies on the kinetics of the appearance of the different populations of cells within the tumor and on the differences in host cells found in progressor vs regressor tumors. In addition we will be examining the functional capacities of the cells isolated from the tumor.

One of our main interests is the interrelationship between the various in vitro assays and the tumor immune response in vivo. We will be persuing this area of research by utilizing different in vitro assays to assess the immune response during tumor growth and regression in both normal and immune animals. Among the assays to be employed are ^{51}Cr release cytotoxicity, $^{125}IUdR$ cytotoxicity, production of migration inhibition factor, suppression, growth inhibition, Generation of primary and secondary response in vitro, and antibody-dependent cell-mediated cytotoxicity.

Studies will continue on the specificity of the tumor immune response as measured by different in vitro assays. In the area of T cell-mediated cytotoxicity we will use techniques such as 4 hour and 18 hour ^{51}Cr release cytotoxicity assays, inhibition of ^{51}Cr release cell-mediated cytotoxicity, primary and secondary responses, and monolayer adsorption to better understand the specificity observed with cells from immune animals.

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Annual Report of the Laboratory of Cell Biology

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Somatic Cell Genetics

a) The detection and location of specific neuron antigens that are expressed by neuron x fibroblast hybrid cells have been studied. Rodent neuron x human fibroblast hybrid cells have been obtained that are losing human chromosomes and these hybrid cells have been injected into rabbits, sera collected, and the antisera adsorbed against the human fibroblast parent and the non-neural tissues of the rodent parent. The resultant antisera are reconcentrated and are reacted with various portions of the freshly dissected embryonic nervous system. The freshly dissected fragments or frozen sections of the rodent nervous system are incubated with adsorbed antisera, washed and then reacted with goat anti-rabbit IgG that has been tagged with fluorescine isothiocyanate, then it is washed again and viewed with fluorescence microscopy and/or a combination of phase and fluorescence microscopy. Localization has been observed of fluorescent label on individual neurons, especially in one case on a group in the dorsal spinal cord. It is anticipated that this will prove a valuable and general new method with which to study specificity in the developing nervous system of mammals.

b) Culture of experimentally induced thyroid cell tumors and their normal cell counterparts from the thyroid cell tumors and their normal cell counterparts from the thyroid gland of rats have been under study. We have been able to grow epithelial cells from tumors and normal rat thyroids that are synthesizing rat thyroglobulin. Continuous culture of differentiated cells from the thyroid gland or from thyroid tumors had not been achieved before this work; only short term cell cultures and organ cultures were available for metabolic studies. Our studies presage a great deal of work on the molecular biology and genetics of regulation in the thyroid gland and its tumors.

c) Mutants for antibiotic resistance are being used to study recombination in mitochondrial DNA. In particular, we propose to map the recombination of these resistance markers by making hybrid and cybrid crosses between the closely related subspecies of mice. At the level of the restriction enzyme patterns of Eco Rl and Hin III the standard laboratory inbred strains of mice are indistinguishable from one another. However, we have found that several of the subspecies of M. musculus - notably M.m.molocinous, M.m.castaneous, M.m.domesticus, and M.m.caroli, and closely related species, e.g., M.cervicolor, all have mtDNA restriction sites easily distinguished by both Eco Rl and Hin III. We have previously shown that cRNA-DNAhybridization can be used as a sensitive means to discriminate among the mtDNA sequences of three of these subspecies. It is anticipated that the more complex patterns obtainable by digestion with other restriction enzymes such as Hae III coupled with cRNA-DNA hybridization of these fragments (the Southern technique) will allow for mapping and the definitive demonstration of mitochondrial recombination

in mammals.

In other work with the mitochondrial genetics system we have found that NP-40 extracts of cytoplasmic DNA (presumably considerably enriched for mtDNA) from chloramphenicol resistant cells may be added to sensitive cell strains using the techniques effective in transforming mammalian cells with adenovirus or SV40 DNA or DNA fragments, and that the chloramphenicol resistance phenotype appears with high frequency ($1/10^3$ - $1/10^4$) among the treated cells. Similar extracts from sensitive cells fail to do this. Thus it appears that cytoplasmic DNA extracts from resistant cells are capable of transforming chloramphenicol sensitive cell strains. It is tempting to speculate that what has happened in our experiments is that the portion of the mitochondrial (?) genome which can confer chloramphenicol resistance has either repopulated the sensitive cells' mitochondria or has somehow become integrated (perhaps via recombination) into the sensitive cells' mtDNA and thus achieved true transformation of a mammalian gene.

Rabbit Immunoglobulins

a) Studies of the structural basis of antibody specificity have involved labeling the antibody combining site and localizing the label within the primary structure of the antibody molecule. The results have all shown that the labels are found within the hypervariable sequences in the variable region of the heavy and/or light chains. Using the paired-iodination technique, we have extended our studies to a second rabbit anti-p-azobenzoate antibody and a tyrosyl residue at position 30 in the light chain has been identified as being in the binding site. These studies together with data reported previously for the light chain of an antibody directed toward the p-azobenzoate determinant demonstrate that both the first and third hypervariable regions in the light chains may contribute to the binding site structure. Thus, there are more than one set of combining site sequences complimentary for a given determinant. The antigenic determinants of rabbit IgG include isotypic specificities and allotypic specificities. The heavy chain allotypes a1, a2 and a3, behave as if controlled by three allelic genes at an autosomal locus. Chemical analyses have established that there are approximately 16 residues in the variable regions of heavy chains which correlate with a locus specificities. Direct proof for the localization of an antigenic determinant, however, can be obtained from isolation of peptides carrying a allotypic specificities. An immunopeptide bearing a3 allotypic determinants has been isolated from the heavy chain of an a3 homozygous rabbit. Its molecular weight was found to be 6,000. This peptide is composed of two polypeptide chains linked by a disulfide bond. It covers positions 17-34 and 64-94 of the variable region. Most of these residues are fully exposed on the surface and are far removed from the antibody combining site. Further structural studies are in progress and may help to define the nature of the variable gene markers.

Histocompatibility Antigens

a) Papain-solubilized HL-A antigens have been shown to contain two polypeptide fragments: β_2-microglobulin with a molecular weight of 12,000 and a larger fragment with a molecular weight of about 34,000. The large fragments

isolated from two HL-A preparations carrying different specificities appeared homogeneous both by immunoelectrophoresis and SDS-acrylamide electrophoresis. The carbohydrate content of the large fragment was 12.9% making the molecular weight of the polypeptide chain of the large fragment approximately 30,000. Small but significant differences have been found in the amino acid compositions and tryptic peptide maps of the large fragment containing different specificities. Both HL-A antigen preparations contained the same NH_2-terminal, glycine. Partial amino acid sequence has been obtained for the first 22 residues. With the exception of one possible difference in position 9, the sequences of the two HL-A preparations are identical. This supports earlier suppositions that the two HL-A loci are homologous to one another and must have come from a common ancestral gene. For the mouse H-2, various cultured cell lines, liver and spleen cells have now been chosen as the source of material. A crude membrane preparation is prepared by differential centrifugation according to established procedures. The membranes are digested by a limited papain digestion which solubilizes H-2. The solubilized H-2 is further purified by gel filtration, affinity chromatography and isoelectric focusing. The purified H-2 is shown to contain two components after SDS-gel electrophoresis; one with a molecular weight of approximately 35,000 and a small component with a molecular weight of approximately 12,000. These observations are in agreement with reports from other laboratories in which trace amounts of H-2 have been prepared. Chemical characterization of the purified large fragments is now in progress as well as studies of broad biologic activities; μg amounts of purified $H\text{-}2^a$ antigen are active in skin graft rejection, induction of humoral tolerance. The biologic effects of purified $H\text{-}2^a$ are also being studied in lymphocyte stimulation and inhibition of PE cell migration. Detergent solubilized preparations are also being purified. Membranes from $H\text{-}2^b$ haplotypes have been carried through a number of steps. This material as judged by SDS-PAGE and immunoprecipitation of radiolabeled antigens is now about 20% pure.

b) A highly purified preparation of mouse β_2-microglobulin has been obtained from sodium thiocyanate extracts of liver cell membranes. The first 40 residues have been sequenced. The mouse protein differs only at 9 positions from human β_2-microglobulins. The sequence also has homology to the constant regions of mouse γG2a, most closely to the C_H3 region. These data support the conclusion that the mouse protein is the mouse homologue of human β_2-microglobulin. Mouse β_2-microglobulin in serum has been found to be associated with a substance of approximately 40,000 molecular weight lacking H-2 alloantigenic specificities. The nature of this protein remains at this moment unknown. The possibility that other specificities for example, of the Ia type are indeed carried by this protein, are being actively investigated. This situation resembles the allogeneic effector factor, where both Ia and β_2-microglobulin antigenic determinants have been shown to be present.

c) Another aspect of our work is the analysis of the H-2 antigens on tumor cells. A monospecific antiserum which will detect H-2.33 ($H\text{-}2^b$) was found to be cytotoxic for DMLM 1678 ascites tumor cells of the $H\text{-}2^s$ haplotype. The H-2 molecules or variants seem to be involved in this reaction and not viral proteins. The importance of this observation is obvious, in view of several suggestions that H-2 antigens are important in the lysis of syngeneic cells.

Tumor Specific Antigens

a) The methods used for purification of H-2 antigens were adapted for a study of TSTA of several well-defined systems: SV 40-induced neoplasms (mKSA, SVT2), leukemogenic virus induced neoplasms (RBC-5, FBC-3) and methylcholanthrene-induced sarcomas (Meth-A and MCA-6). Purification procedures have been initiated following solubilization with limited papain digestion; in addition purification following solubilization with detergents (deoxycholate and Triton X 100) is in progress in view of the fact that papain digestion may not be ideal for TSTA.

Quantitative studies have been carried out on the immunogenicity (tumor rejection) of purification fractions of the mKSA sarcoma (SV 40-induced). Assessment was made through the use of dose-response curves. Despite the fact that only 2-3% of TSTA activity was recoverable in the papain-solubilized fraction, immunization in syngeneic mice was achieved with 2 μg of this material; tumor size (expressed in terms of frequency and tumor volume) was reduced to 30% of controls. The high activity of the solubilized material was also observed in specific lymphocyte stimulation (1 μg was optimal) and specific inhibition of PE cell migration (activity was achieved with nannogram amounts.) Fractionation of the solubilized material, in contrast to H-2 antigens, by chromatography on DEAE-cellulose, Sephadex-G 150 and Concanavalin A-Sepharose columns did not concentrate immunogenic activity. It may be that SV-40 TSTA is susceptible to extensive degradation by papain - although other explanations for loss of activity are possible. We have no indication as yet that solubilization by other methods is superior.

Studies of attempts at purification of SV-40 TSTA provide some comparative information concerning the relationship of H-2 and TSTA: 1) As mentioned there is a distinct difference in sensitivity to papain of H-2 and the (SV40)TSTA; 2) H-2 elutes in a sharp peak during gel filtration whereas our specific TSTA elutes in more than one peak; 3) After affinity chromatography, H-2 activity was found in the bound Con A II fraction whereas specific TSTA activity was found in the unbound Con A I fraction.

T-antigen, a nuclear antigen also induced by an "early" SV40 virus gene and detectable by CF assays has been proposed to provide tumor-rejection activity. Our highly immunogenic fraction (Crude Soluble) as well as DEAE I and G150 I fractions having tumor rejection activity did not contain measurable T-antigen activity.

Of special interest is the observation that SV40 TSTA of the Syrian hamster (tumor SV 34 of Takemoto) and mKSA of BALB/c origin cross react. Solubilized TSTA from mKSA has the capacity to induce tumor rejection in the hamster and solubilized TSTA from SV 34 has the capacity to induce specific tumor rejection in BALB/c mice against mKSA. Cross-reactivity was also observed in inhibition of PE cell migration where as low a concentration as 0.00025 μg/ml of either TSTA was effective. In addition, in collaboration with Dr. Kenneth Takemoto some of the immune properties of hamster neoplasms induced *in vitro* by the human papovaviruses BK and JC have been studied. These neoplasms have T antigen and T antisera that cross-react with T-antigen of an SV40-induced hamster sarcoma (SV-34). Although both types of neoplasms

induced by the BK and JC viruses share T-antigen no evidence for the existence of TSTA could be found.

b) The TSTA of Meth A sarcoma of BALB/c mice induced by methylcholanthrene has been solubilized both by Triton X and by papain. The tumor rejection capacity of this TSTA per μg protein is much lower however than that observed for the mKSA neoplasm.

c) We have continued our studies of the "eclipse" phase of growth of our mKSA (SV 40-induced) neoplasm. BALB/c mice bearing large tumors (< 15mm in diameter) are unable to reject this syngeneic tumor and T cells from these tumor bearers are not competent as seen in the Winn assay; nevertheless, 10 days following amputation of the tumor, the animal is now immunologically competent and T cells will then neutralize tumor cells in a specific fashion (Winn assay). We have found a fraction (fraction II) in ascites fluid and in serum of tumor bearing mice that will 1) extend the "eclipse" period in amputated mice and will 2) also provide progressive growth of mKSA (Tu 5) cells, tissue culture-propagated cells that have lost their oncogenic potential. This factor was found also in DBA/2 mice bearing L1210 ascites but not in ascitic fluid of several other ascitic tumors: FBL-3 and plasma cell neoplasms (syngeneic with mKSA). Fractionation studies are in progress to define this factor.

RNA Virus-Induced Neoplasms

d) The nature of the virus-determined cell surface antigens (CSAs) and of TSTA and of their interrelations and of the role of virion components P30, P15, GP 69/71 found on leukemic cells is a problem receiving much emphasis. A chromatographed fraction (F2 from G-150 Sephadex) was shown previously to provide activity in a ^{51}Cr-release cytotoxicity assay and to provide specific tumor rejection of FMR type leukemias. Through the use of a CF assay it was found that a specific component presumably not virion antigens existed in our F2 fraction that specifically lysed RBL-5 cells.

e) Since we now have the purified virion components P30 and GP 69/71 and antisera to those components (from Dr. S. Oroszlon, Flow Labs) we are in the process of determining their role in the specific cytotoxic response employing our syngeneic antisera to membrane components and their role in tumor rejection, that is the presence of TSTA. We have found a) that P30 and GP70 are not responsible for cytotoxicity with our syngeneic antiserum and b) that they do not play a role in tumor rejection (using FBL-3 and RBL-5) in syngeneic B6 or semi-syngeneic CBF_1 mice. Solubilized antigen that has had virion components P30 and GP69/71 removed by passage through columns containing anti P30 and GP69/71 retain their capacity to immunize specifically as measured by tumor rejection.

H-2j

H-2K and H-2D Loci and Tumor Cell Kill

a) Two groups of investigators have now reported that leukemic cell targets are lysed, as detected in a cell-mediated ^{51}Cr assay only when effector cells and target cells are syngeneic at the H-2K and/or H-2D loci. The most likely proposed mechanism is an alteration of the H-2 molecule or an

interaction occurs between a viral and an H-2 molecule. The TAA in each case is not known nor are TSTA present in the systems investigated. More recently it has also been postulated that the H-2 type of cell used for immunization is also a decisive factor in the T-cell killers generated. We are presently investigating whether a) this phenomenon is characteristic only of the ^{51}Cr-release assay - by using an IuDR assay employing other than RNA virus-induced neoplasms and also investigating the in vivo significance of H-2 sharing employing matched and unmatched loci in the Winn assay.

In Vitro Studies of Cell-Mediated Immunity

a) Specific afferent interference by antiserum of in vivo tumor immunity: We have studied the effect of various antiserum, whose specificity have been well characterized, on the immunogenicity of FBL-3 cells to induce in vivo transplantation immunity. The antisera used in these experiments included: anti Friend leukemia sera, anti SV40 tumor sera, and anti fetal sera. It was found that induction of in vivo tumor immunity could only be specifically blocked by the antiserum with appropriate antigenic specificity. These studies not only showed that antibodies could play some role in in vivo immunity, but also offered a simpler method to define the specificity of tumor associated transplantation antigen.

b) In vivo and in vitro induction of cell-mediated cytotoxic responses: We have modified the IRA for use with suspension target cells to measure the cell-mediated cytotoxic responses to FBL-3 cells, a Friend virus induced leukemia in C57BL/6 mice. By employing this technique, we have been able to detect the primary and secondary cell-mediated cytotoxic response both by in vivo immunization or challenge or by in vitro mixed lymphocyte tumor cells culture technique. The secondary response could be easily distinguished from the primary response in several aspects: it was enhanced (gave higher levels of cytotoxicity), accelerated (peaked at earlier times) and longer lasting. The characteristics of these cytotoxic responses gave a very good correlation to the in vivo tumor immunity: specificity of the reaction, characteristics of the lymphocyte population involved, relationship to the host immune status (progressors vs. regressors) etc. Thus, it appears that we can use these techniques to study the mechanisms for the induction of in vivo tumor immunity.

c) Humoral control of tumor growth and immune response: We have found that some humoral factors are produced in tumor bearing hosts that could affect the tumor growth and the host immune response. This is based on the following observations: 1) The tissue fluids obtained from tumor bearing hosts in mouse and man could promote the subcutaneous growth of FBL-3 cells in syngeneic hosts. These tumors usually regressed without the tissue fluids administration; 2) These tissue fluids were able to interfere with the effector phase of cell-mediated cytotoxic reactions (efferent interference); 3) Some of these tissue fluids were able to suppress the induction of cell-mediated cytotoxic responses (afferent interference), thus, it was evident that tumor growth promoting factor(s) (GPF) and immunosuppressive factor(s) (ISF) were present in the tumor bearing hosts. In addition, α-globulin was also found to be able to inhibit the cell-mediated cytotoxic reaction.

d) Characterization of soluble antigens: Various soluble antigens have been used to study their effects on the inhibition of cell-mediated cytotoxic reactions to FBL-3 cells. It was found that soluble antigen obtained from FBL-3 cells could give complete inhibition, whereas the H_2 antigens prepared in similar fashion did not. In addition, the group specific antigen gp70 could give partial inhibition, whereas p30 was not able to do so. These findings indicated that various antigens might be responsible for the induction of cell-mediated immunity, as well as in vivo tumor immunity. Further studies will be carried out to study the specificity of these reactions, and the effect of soluble antigen on the induction of cell-mediated cytotoxic responses.

Intracisternal A Particles

a) It has been previously established that intracisternal A particles (IAP) have several biochemical characteristics known to be associated with murine oncornaviruses, namely high molecular weight RNA and reverse transcriptase. It has also been shown that IAP can be detected in the two-cell embryo, suggesting that vertical transmission can occur. However, until now there has been no evidence that IAP can be transmitted horizontally; demonstration of this capability would lend credence to the concept that IAP have infectious properties. Through the use of techniques for somatic cell hybridization, we have been able to transfer IAP from enucleated IAP-positive cells (cytoplasts) to IAP-negative cells by fusing them in the presence of inactivated Sendai virus. Such cytoplast-whole cell hybrids are designated as cybrids. By using mutant markers the identification of both parent lines and the resulting cybrids could be achieved. IAP-positive BALB/c mammary tumor tissue culture cells (MT) were mutagenized and selected for resistance to chloramphenicol (CAP) and ouabain while 3T3-4EF cells (IAP-negative) were selected for resistance to BUdR. As a preliminary experiment it was determined that the CAP resistance could be transferred in the cytoplasm by fusing the mammary tumor cytoplasts to sensitive cells. MT cytoplasts were then fused to 3T3-4EF cells and selected in CAP and BUdR. The resulting cybrid clones were resistant to CAP and BUdR and sensitive to ouabain, and had marker chromosomes and the phenotype of 3T3-4EF cells. 80% of the clones showed continued IAP production over some 60-90 cell generations. These results suggest that the genetic information coding for IAP can be transferred in the cytoplasm and thus horizontal transmission can occur under appropriate conditions. The cybrid cell lines represent an important development for studying the mode of replication of IAP, i.e., they can be used to answer the following questions: Does fusion allow for establishing an infection with IAP or does it activate an IAP genome already in the recipient cell? Appropriate nucleic acid hybridization experiments should clarify this situation. If the parent 3T3-4EF line does not contain IAP genes and the cybrid does, then it is probable that infection has taken place. However, if the 3T3-4EF cells do contain IAP genes, then fusion may have served to activate a pre-existing genome. Since it is now possible to delineate this potential for infectivity, a major question regarding the ultimate identity of IAP as viruses can be answered.

b) Neuroblastoma cells and C-type virus expression. Although there have been unconfirmed reports that C-type viruses can cause neuroblastomas in strain A mice, there is no unequivocal evidence that such viruses cause any neoplastic lesions other than leukemias and sarcomas. In an attempt to clarify some of the relationships between MuLV and neuroblastoma, we studied three tissue culture clones derived from the C1300 tumor. None of the clones released endogenous ecotropic virus spontaneously, but one did release xenotropic virus. Two of the clones could be induced to release virus when treated with IUdR and dexamethasone, but one clone, N-18 did not release either ecotropic or xenotropic virus under a variety of induction conditions. All three clones showed evidence of gs-1 antigen as measured by immunofluorescence. Although clone N-18 did not release virus, these tissue culture cells were still capable of producing tumors in the mouse. Thus the ability to control virus production was independent of the oncogenic potential. Clone N-18 provides an excellent in vitro system for studying factors that regulate C particle gene expression, particularly since these cells are derived from a mouse strain with low leukemia incidence.

c) Ultrastructure of plasma cell tumors and lymphosarcomas. It is now apparent that Abelson MuLV can act as a co-carcinogenic agent in concert with Pristane to rapidly induce plasma cell tumors in BALB/c mice. However, some of the tumors develop before differentiation into plasma cells is complete, that is, there is a whole spectrum of neoplasms ranging from lymphosarcomas to intermediate forms to classical myelomas. We have begun to better characterize this system by comparing the tumor ultrastructure with the ability to secrete immunoglobulin and produce IA antigen. A representative survey of several tumors indicates that the secretory products are found only in those cells which can be classified as plasma cells or as lymphocytes with a fairly extensive rough endoplasmic reticulum and a prominent Golgi complex. Those tumors containing only small lymphocytes with free ribosomes appear to be unable to secrete either immunoglobulin or IA antigen.

Transfer RNA Studies

a) A structural difference between an additional Phe-tRNA (Phe-tRNA$_1$) found in 3 rat hepatomas and that Phe-tRNA (Phe-tRNA$_2$) found in normal rat liver and tumors has been described. This difference seems to involve the absence of a very highly modified, hydrophobic and fluorescent base called "Y" in Phe-tRNA$_1$ which is present in Phe-tRNA$_2$. Repeated study of chromatographic profiles of Phe-tRNA from a variety of rodent tumors has shown that Phe-tRNA$_1$ may really consist of two peaks which are so similar that they are hard to separate chromatographically. Both these peaks are seen in small amounts in the following rat tumors: Morris hepatomas 5123D, 7777, and 7800, Sarcoma LA-2, Walker mammary Ca. 256, Thyroid Cas. 1-2 and 1-6. Unusually large amounts of these human-specific Phe-tRNAs were found in the spontaneous rat plasmacytoma IR-162 and the Gross virus induced thymoma, French NTD. Negligible amounts of these tumor-associated molecules were found in Morris hepatoma 5123, spontaneous mammary Ca. 4M, and basophilic leukemia RBL-1. A variety of mouse plasmacytomas and spontaneous mouse hepatomas failed to show any evidence of tumor specific Phe-tRNA.

b) Mouse Plasmacytoma tRNA Comparisons

Several lines of BALB/c plasmacytomas were selected for tRNA studies because of the nature of the immunoglobulin they secreted. Two tumor lines secreting only kappa type light chains with identical amino acid sequences except at two positions in V_L, two lines secreting IgAs with identical light and heavy chains except for a single V_H residue difference and three IgA lines with identical C_L, V_L and V_H but different C_H regions were studied. Two cloned lines of IgG producing tumors were compared with two non-producing daughter clones which spontaneously arose during tissue culture. So far the tRNAs for 11 of the 20 natural amino acids have been examined for these tumors as well as for another tumor grown with and without treatment with BUdR. Until the remainder of the 20 amino acids have been studied our conclusions must be tentative and preliminary, but so far no definite correlation between particular isoaccepting tRNAs and sequence differences in immunoglobulins secreted has emerged. There are striking differences in certain isoaccepting patterns, particularly Ser-tRNA and striking lack of differences among others, notably Gly-, Asp- and Leu-tRNAs.

c) IgA Half Molecules (IgA_h)

Direct comparison of the molecular size of six of the above described IgA_h myeloma proteins by polyacrylamide gradient gel electrophoresis shows that all six appear as L_1H_1 molecules of identical size, ca. 65,000 daltons, apparently the smallest immunoglobulins so far reported. In addition, all six have heavy chains of the same size, ca. 40,000 daltons, which would be the smallest immunoglobulin heavy chains known. Since proteolytic generation of these unusually small molecules from larger ones has been ruled out by our earlier intracellular kinetic studies, and since chance chain terminating mutations are unlikely to generate 6 molecules of identical size, we feel that these represent a second IgA subclass in mice. Homologous class-specific antiserums were prepared for IgA_h, and they detected immunologic evidence of IgA_h in normal BALB/c mouse gut contents. This is additional evidence that IgA_h is a normal IgA subclass in mice.

Genome Rescue and Characterizations of a Rat Hepatoma Helper Virus (Pseudotype Kirsten Sarcoma Virus)

A major effort in this laboratory is concentrated toward a better understanding of neoplastic transformation by adopting as an experimental model system the chemically induced rat hepatoma, which has been adapted to in vitro growth in tissue culture, called HTC cells. HTC cells are heteroploid rat tumor cells established from an ascitic form of the rat solid hepatoma 7288c originally induced in 1962 by the chemical carcinogen, N,N-2,7-fluorenylene-bis-2,2,2-trifluoroacetamide. Biochemical properties, principally those involved in corticosteroid responses and some aspects of cytogenetics of HTC cells have been well studied. These included the induction of tyrosine aminotransferase, altered cell surface characteristics, and specific increases in one iso-accepting form of phenylalanyl transfer RNA as a result of dexamethasone phosphate (DXP) treatment of HTC cells. These facts, together with the augmentation of virus induction in mouse systems by DXP, led us to attempt

similar experiments in HTC cells. However repeated attempts of virus induction in a few HTC lines including HTC-H1, treated with either 5-iodo-deoxyuridine (IUdR) or 5-bromo-deoxyuridine (BUdR) at varying dosages optimal for induction of C-type viruses in the mouse systems, with DXP, failed to elicit increased level of reverse transcriptase activity as compared to untreated HTC cells. Neither was the release of infectious C-type virus observed in these chemically treated HTC cells when conditioned media from these cultures were assayed for cell transformation activity. Recently extensive efforts in genome rescue experiments resulted in the isolation of a rat hepatoma virus pseudotype Kirsten sarcoma virus, KiMSV(RHHV), when HTC-H1 cells were co-cultivated with Kirsten sarcoma virus transformed non-producer rat kidney cells (K-NRK). KiMSV(RHHV) is now cultured at a high titer (10^4 focus forming units/ml of medium) in vitro. Biological, morphological, biochemical and immunological characteristics of KiMSV(RHHV)are currently under investigation. It has a C-type virus ultra-structure. It is strictly rat tropic in host range. The helper virus, RHHV, originates from the hepatoma cell as suggested by the mode of its rescue and by its host specificity. Nucleic acid hybridization analysis is now carried out to further substantiate this point. Results obtained by indirect fluorescent-antibody assays and neutralization bioassays indicate that KiMSV(RHHV) exhibits a major rat leukemia virus (RaLV) specific antigen (GP 70, glycoprotein of 70,000 mol. wt.) and an inter-mammalian group specific gs-3 (P30, 30,000 mol. wt. protein) antigen. The major viral protein associated with KiMSV(RHHV), when the virus was labelled in either ^{35}S-methionine or ^{3}H-amino acids and then analyzed by polyacrylamide gel electrophoresis, is a 30,000 mol. wt. protein. A very minor radioactive band can also be consistently detected at the 70,000 mol. wt. area; whether this 70,000 mol. wt. material represents a membrane glycoprotein that accounts for the neutralization specificity of RaLV or enzymatic components of the virus remains to be resolved. We are now attempting to radioactively label KiMSV(RHHV) at a higher specificity so that other minor protein fractions may also be detectable. KiMSV(RHHV) sediments isopycnically in a linear sucrose gradient at 1.145-1.155 g/cm^3, less dense than the known mouse C-type tumor viruses. It also possesses the complements of RNA and reverse transcriptase. Its possible tumorigenic and immunogenic effects in vivo are under investigation in a long-termed project. We have observed so far that when KiMSV(RHHV) transformed Fisher rat embryonic cells were inoculated into syngeneic new-born or young adult rats, not only was there rapid growth of a capsulated tumor at the site of inoculation, but metastases were also seen primarily to the liver.

New Lymphomas for Study of Tumorassociated and Virion Antigens

a) To distinguish between viral structural antigens and tumor specific transplantation antigens (TSTA) expressed on the membranes of a type-C virus-induced tumor, RBL-5, which was originally induced by Rauscher-MuLV, (R-MuLV), and is still producing the virus, has been used in recent years. The advantage of using another tumor, RBL-3, which was originally induced by R-MuLV but found to produce neither infectious type-C viruses nor mature virion particles by electron microscopy is obvious. This tumor was established as a culture. The presence of R-MuLV genome in RBL-3 is evidenced by the emergence of infectious R-MuLV after treatment of these cells with 200 mcg/ml 5-iododeoxyuridine (IUdR), or repeated passages in vivo. The cells contain gp69/71, 20-26 ng/10^6 cells, and p30 10-31 ng/10^6 cells as detected by radioimmunoassay, and are gs-positive by complement fixation test.

RBL-3 also exhibited FMR group transplantation antigen. B6 mice immunized with RBL-3 were resistant to challenge with FBL-3 leukemia induced originally by Friend-MuLV, and vice versa; (BALB/c x C57BL/6)F_1 mice immunized with RBL-3 were resistant to challenge by another nonproducer tumor (HFL/d-tc) derived from Friend-MuLV-induced tumor in BALB/c mice (Friedman and Lilly). Furthermore, the crude membrane (CM) of RBL-3 and crude soluble (CS) antigen prepared from the former by limited papain digestion induced immunity in B6 mice against FBL-3 challenge. The CM contained both gp69/71 and p30, but the CS contained no p30. Further work is in progress to purify the TSTA from RBL-3.

The cell membrane antigens present on RBL-3 are complex. By membrane immunofluorescence studies with appropriate antisera we could demonstrate the presence of gp69/71, p30, and an antigen or antigens (presumably non-virion tumor antigen) which reacted only with the syngeneic antisera prepared against the CM of RBL-3. Although this antiserum contained anti-gp69/71 and anti-p30, the activity against RBL-3 surface antigen could not be removed by repeated absorption of this serum with unrelated lymphoid tumor cells such as plasmacytoma C1.18(4) cells expressing these virion ants. This syngeneic anti-RBL-3-CM serum cross-reacted with RBL-5 to a certain extent but still retained its specific activity against RBL-3 even after absorption of the serum with RBL-5. Similarly, the syngeneic anti-RBL-5-CM serum cross-reacted with RBL-3 only at low dilutions. These reactivities were not removed by absorption with B6 fetal tissues. The nature of these RBL-3 specific and RBL- specific antigens needs further investigation.

Thus, in the present system, by using syngeneic anti-CM sera and anti-gp69/71, anti-p30 or broad reacting antiviral sera in parallel, and by using complement fixation, cytotoxicity, and membrane immunofluorescence tests, it has been shown that a tumor specific antigen(s) exists which is distinct from gp69/71, p30 or other virion antigens detectable by these antiviral sera. The identity of this tumor specific antigen with TSTA needs to be established in the future.

It was also found that RBL-3 cells possess Fc receptors as evidenced by EA rosette formation, and their ability to act as effector cells for antibody-dependent-cell-mediated cytotoxicity against ^{51}Cr-labeled sheep RBC. Furthermore, kappa chain of Ig was found on the cell surface as detected by membrane immunofluorescence. The tumors formed by the cell transplant in B6 mice produced reticulum fibers, and were classified as RCN-A.

Changes in Cell Membrane Components in Exponentially Growing Mouse Fibroblast Cells

a) A family of mouse fibroblast cell lines including normal, spontaneously and SV40 virus transformed lines, and clones and subclones thereof, were compared in exponential phase of growth in protein constitution of their cell membranes. By monitoring for specific (enzymatically removable) cell surface components, such as labelled sialic acid, we developed a method to obtain preparations from these cells enriched in cell surface membrane. We observed one (glyco-) protein component (apparent mol. weight 180,000 as characterized by acrylamide SDS gel electrophoresis and autoradiography) to be reduced or absent in an SV40 virus transformed mass cell line when compared

with the normal mass cell line. No such compositional difference was observed using protein staining only in a spontaneously transformed tumorigenic clonal derivative cell line, or in sister subclones of such derivative cell line with or without SV40 virus infection. However, in metabolic labelling experiments with ^{14}C mixed amino acids a consistent decrease also was demonstrated in the biosynthesis of the same (glyco-)protein in the SV40 virus infected subclones, as compared to uninfected sister subclones. In the cell lines investigated no correlation was discernable between the membrane (glyco-)protein constituent in the exponentially growing cells and tumorigenicity or saturation density. Apparently the cells ability to grow to high density in monolayers in attachment, and also the tumorigenicity is not a simple correlate of the protein change in the membranes, as detectable by the techniques employed. However, the ability of the SV40 transformed cells to grow well without attachment in viscous suspension in methocell relates to the observed membrane differences, that is to the reduced amount of the 180,000 molecular weight (glyco-)protein constituent.

Previous studies by others showed differences in high molecular weight proteins in confluent or post confluent cell layers between untransformed and virally transformed fibroblastic murine cell lines, or cell lines transformed by temperature sensitive viruses grown at the permissive temperature vs. those grown at non-permissive temperature. Such differences are now known to be attributed to the fact that untransformed cells became arrested in a G_1 (or G_o) phase of growth when reaching confluence in a monolayer, but the (virally) transformed cells continued to divide. Our observation of differences in protein patterns and in metabolic labelling in exponentially growing cells pertain to the presence and functioning of SV40 genes (as expressed by production of T and tumor specific surface antigens), and to our knowledge it is the first which directly correlates membrane protein changes with SV40 transformation and not with indirect effects of serum and hormonal factors in the different stages of growth. There were no previous studies at all on the membranes of spontaneously transformed tumorigenic cells to contrast them with normal or virally transformed related cells.

Pathogenesis of Plasma Cell Tumor Development

a) Susceptibility to oil-induced plasmacytoma development in BALB/c is multifactorial. The strain dependence however suggests that specific genes can be identified. The differences between BALB/c and the resistant strain C57BL/Ka is being studied.

Analysis of the Bailey RI strains derived from crosses between BALB/c and C57BL/6 revealed two strains that were similar to BALB/c: CXBG and CXBJ. The only genes these mice had in common that were absent in the other five RI strains were the IgC_H allotypes that resembled those in BALB/c. The allotype locus in the mouse is known to be linked to V_H genes and thus the influence of the immunoglobulin structural genes in plasmacytoma susceptibility is suggested. Currently the susceptibility of F_1 hybrids of a^1 a^2 (C57BL/Ka x BALB/c) x C57BL/Ka mice segregating for allotypes a^1 and a^2 will be tested for susceptibility to pristane induced plasmacytomagenesis.

b) If BALB/c susceptibility to plasmacytoma development is dependent in part on a genetic property of a gene exposing itself in the B-lymphocyte-plasma cell lineage, then this phenotype could be augmented by somatic mutations. Assuming a 2-hit genetic change one coded by a germ-line gene the other augmented by a somatic event, it should be possible to increase plasmacytoma development, by exposing BALB/c mice to mutagenic agents. In early pilot studies using Ethylmethane Sulfonate plasmacytoma development was accelerated. Attempts to extend, amplify, and coordinate this finding with other mutagens are still in progress; currently Ethylnitrosourea is being tested. In two experiments in progress, a more discriminatory protocol is being tested in which ENU treatment is given before injection with 0.5 ml pristane. Thus far, ENU is producing lymphomas (predominantly T-lymphomas) in BALB/c mice, but has not produced an increase in plasmacytomas. In these experiments care must be taken to avoid toxicity, which can in itself modify susceptibility.

c) MLV-A virus contains a complete replicating unit, the Moloney Leukemia Virus, and a replication defective component which is responsible for the transforming properties of the virus. MLV-A is capable of infecting adult BALB/c mice and inducing non-thymic lymphoreticular tumors very rapidly i.e. within 21 to 100 days. We are continuing to characterize the tumor types in BALB/c mice and other related strains of mice, the Bailey RI strains. When the virus is injected into the peritoneal cavity of mice that have previously received an injection of pristane, the principle neoplastic type obtained are undifferentiated lymphosarcomas (LS) which are able to synthesize and produce small amounts of immunoglobulin, chiefly IgM that is deposited in the cell membrane. This group of tumors comprises 80% of all the tumors produced in BALB/c. In addition there are more differentiated tumors of the immunoglobulin producing series including plasmacytomas and plasmacytoid-lymphosarcomas (PL) (probably a cell quite similar to the chronic lymphocytic leukemia cell in man) in its level of differentiation. We are currently attempting to develop a method for rapidly characterizing these lymphocytic tumor types so that lymphocytic tumors can be classified. Preliminary studies indicate that the PL tumors have more immunoglobulin on their surface than the LS tumors. LS and PL tumors are being screened by the rosette method for their ability to bind antigens; SRBC coupled with phosphorylcholine, galactan, bacterial levan, S. tranoroa lps, TNP and dextran are being used as probes. These activities are the most common antigens involved in BALB/c myeloma protein binding.

d) In collaboration with Rose Lieberman a new genetic marker linked to the IgC_H locus has been identified LEV2,1.1. The strain distribution of this marker is unique thus far, and will provide a valuable new marker that is easy to identify. The idiotype is cross specific and is found on most B2,1 binding myeloma proteins and on antibodies raised to Aerobacter levan. It is probably a natural antibody in most strains of mice. The absence of the marker in the C57BL strain permitted the establishment of linkage to IgC_H by its characteristic distribution in RI strains and congenic strains.

Immunochemistry of Myeloma Proteins

a) Previous studies in this laboratory have involved the determination of the primary and three dimensional structure of the antibody combining site of a phosphorylcholine binding myeloma protein, M603. Comparative primary structural analysis has also been performed on several other phosphorylcholine binding proteins all of BALB/c origin. Having determined the structure of phosphorylcholine antibodies in BALB/c mice we are currently attempting to extend this study to analyze induced anti-phosphorylcholine antibodies in other mouse strains as well as other species. Preliminary data suggest that certain other mouse strains make the same anti-phosphorylcholine antibodies as those found in the BALB/c mouse. These findings have important implications for proposed theories of antibody evolution and diversity and should continue to shed light on the mechanisms involved.

b) Having determined the three dimensional structure of the M603 protein we are now attempting to perform similar experiments on proteins with other binding specificities. In this regard crystals have been obtained from the Fab fragments of J539 and CBPC4, two galactan binding proteins and EPC109 a levan binding protein. Data collection has been made for the J539 protein and a low resolution x-ray map should be available in the near future. To permit construction of a three-dimensional model of this, protein studies to determine the amino acid sequence are in progress. Approximately 60% of the light chain sequence has been completed including two of the three hypervariable regions which are known to contribute to the antigen binding site. Studies on the heavy chain have defined about 90% of the variable region sequence. These data along with future high resolution x-ray analysis will permit the construction of a molecular model and the comparison with the M603 structure.

Studies are also in progress to determine the amino acid sequence of several other galactan binding proteins. We intend to ascertain if the type of variation seen among phosphorylcholine binding proteins is also true for galactan binders. Again, this should provide a further understanding of the mechanisms involved in the generation of antibody diversity.

c) A final group of proteins selected for comparative structural studies are a series of immunoglobulins which react with levan containing antigens. More than ten of these have been identified and members of this group have been shown to possess shared antigenic determinants (idiotypes) in their variable regions. To date, nearly complete sequences have been obtained from the heavy chains of three of these proteins. Experiments are currently being conducted to provide the methods for isolation of the appropriate peptides from the light chains and their subsequent sequencing. This set of proteins will provide data involving a third binding specificity and thus hopefully permit a final analysis of variation of structures among related antibodies.

The studies described have led to a continuing attempt at the in vitro synthesis of antibody molecules with M603 as a model. An understanding of the primary and three dimensional variations of antibody molecules by

studies such as those described may eventually permit the in vitro design and synthesis of antibodies with pre-determined specificity.

d) We are continuing to produce additions to the collection of myeloma proteins that increase the number of examples with a similar binding activity. There were two new B2,1 fructosan binding proteins TEPC957 bringing the list of transplantable tumors in this group to 7. These proteins are being produced in large quantity for sequencing by Drs. Mark Vrana and Stuart Rudikoff. The ABC48 B2,6 fructosan binding protein has been isolated by affinity chromatography in collaborative studies with Dr. Glaudemans and has also been provided for sequencing.

Alpha 1,6, dextran binding proteins have been isolated by affinity chromatography and the L and H chains separated. L and H chains of QUPC52 and W3129 are being provided for sequencing studies. These two proteins differ remarkably in the size of their binding sites. Sequences of these proteins should provide data for constructing hypothetical 3 dimensional models and confirmation of the predictions by Cisar and Kabat on the differences in the topography of the binding sites.

In collaborative studies with Dr. Davies' group proteins have been provided for crystallization studies. Three new proteins have been crystallized and will possibly be useful in establishing electron density maps: the B2,1 fructosan binder EPC109, and the galactan binders CBPC4 and TEPC601.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CBD 03200-08 LCBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
A Study of the Various Factors Influencing the Induction, Growth and Repression of Primary and Transplantable Neoplasms (Host and Tumor Cell Variables)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. W. Law	Chief	LCBGY	NCI
OTHER:	E. Appella	Medical Officer (Res.)	LCBGY	NCI
	M. Rogers	Senior Staff Fellow	LCBGY	NCI
	O. Henriksen	Senior Staff Fellow	LCBGY	NCI
	T. Natori	Expert	LCBGY	NCI
	J. L. McCoy	Investigator	Bionetics	
	J. Dean	Investigator	Bionetics	
	R. Ting	Investigator	Biotech	
	K. Takemoto	Virologist	LVD	NIAID

COOPERATING UNITS (if any)
Bionetics
Biotech Research Laboratories
NIAID

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.25	1.75	4.50

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives: Major emphasis is placed upon the study of tumor antigens of the transplantation rejection type (TSTA) and of the immune responses they evoke. As a corollary to this study the biologic properties in vitro and in vivo of Histocompatibility (H-2) antigens are under study. Solubilization and methods of purification of both TSTA and H-2 are under investigation with the ultimate purpose of defining these membrane antigens in physico-chemical, biologic and molecular terms.

Histocompatibility Antigens

Further purification of H-2 antigens from cell membranes of dissociated spleen cells and liver cells of $H\text{-}2^a$ and $H\text{-}2^b$ haplotypes is continuing (see Annual Report of Appella). In the case of $H\text{-}2^a$ antigen purification, a degree of 80-90% purification (600- fold) has been achieved using a combination of gel filtration, affinity chromatography, and discontinuous polyacrylamide-gel electrophoresis following limited papain digestion. Ample purified material is now available for amino acid sequencing and for defining the purified material in terms of its broad biologic activities; µg amounts of purified $H\text{-}2^a$ antigen are active in skin graft rejection ($H\text{-}2^b$ mice), induction of humoral tolerance ($H\text{-}2^d$ mice). The biologic effects of this material in lymphocyte stimulation and in inhibition of peritoneal cell migration are under study.

Tumor Specific Antigens

1. The methods used for purification of H-2 antigens were adapted for a study of TSTA of several well-defined systems: SV 40-induced neoplasms (mKSA, SVT2), leukemogenic virus induced neoplasms (RBC-5, FBC-3) and methylcholanthrene-induced sarcomas (Meth-A and MCA-6). Purification procedures have been initiated following solubilization with limited papain digestion; in addition purification following solubilization with detergents (deoxycholate and Triton X 100) is in progress in view of the fact that papain digestion may not be ideal for TSTA.

2. a) Quantitative studies have been carried out on the immunogenicity (tumor rejection) of purification fractions of the mKSA sarcoma (SV 40-induced). Assessment was made through the use of dose-response curves. Despite the fact that only 2-3% of TSTA activity was recoverable in the papain-solubilized fraction, immunization in syngeneic mice was achieved with 2 µg of this material; tumor size (expressed in terms of frequency and tumor volume) was reduced to 30% of controls. The high activity of the solubilized material was also observed in specific lymphocyte stimulation (1 µg was optimal) and specific inhibition of PE cell migration (activity was achieved with nannogram amounts. Fractionation of the solubilized material, in contrast to H-2 antigens, by chromatography on DEAE-cellulose, Sephadex-G 150 and Concanavalin A-Sepharose columns did not concentrate immunogenic activity. It may be that SV-40 TSTA is susceptible to extensive degradation by papain - although other explanations for loss of activity are possible. We have no indication as yet that solubilization by other methods is superior.

Studies of attempts at purification of SV-40 TSTA provide some comparative information concerning the relationship of H-2 and TSTA: 1) As mentioned there is a distinct difference in sensitivity to papain of H-2 and the (SV40)TSTA; 2) H-2 elutes in a sharp peak during gel filtration whereas our specific TSTA elutes in more than one peak; 3) After affinity chromatography, H-2 activity was found in the bound Con A II fraction whereas specific TSTA activity was found in the unbound Con A I fraction.

T-antigen, a nuclear antigen also induced by an "early" SV40 virus gene and detectable by CF assays has been proposed to provide tumor-rejection activity. Our highly immunogenic fraction (Crude Soluble) as well as DEAE I and G150 I fractions having tumor rejection activity did not contain measurable T-antigen activity.

Of special interest is the observation that SV40 TSTA of the Syrian hamster (tumor SV 34 of Takemoto) and mKSA of BALB/c origin cross react. Solubilized TSTA from mKSA has the capacity to induce tumor rejection in the hamster and solubilized TSTA from SV 34 has the capacity to induce specific tumor rejection in BALB/c mice against mKSA. Cross-reactivity was also observed in inhibition of PE cell migration where as low a concentration as 0.00025 μg/ml of either TSTA was effective. In addition, in collaboration with Dr. Kenneth Takemoto some of the immune properties of hamster neoplasms induced in vitro by the human papovaviruses BK and JC have been studied. These neoplasms have T antigen and T antisera that cross-react with T-antigen of an SV40-induced hamster sarcoma (SV-34). Although both types of neoplasms induced by the BK and JC viruses share T-antigen no evidence for the existence of TSTA could be found.

b) The TSTA of Meth A sarcoma of BALB/c mice induced by methylcholanthrene has been solubilized both by Triton X and by papain. The tumor rejection capacity of this TSTA per μg protein is much lower however than that observed for the mKSA neoplasm.

c) We have continued our studies of the "eclipse" phase of growth of our mKSA (SV 40-induced) neoplasm. BALB/c mice bearing large tumors (15mm. in diameter) are unable to reject this syngeneic tumor and T cells from these tumor bearers are not competent as seen in the Winn assay; nevertheless, 10 days following amputation of the tumor, the animal is now immunologically competent and T cells will then neutralize tumor cells in a specific fashion (Winn assay). We have found a fraction (fraction II) in ascites fluid and in serum of tumor bearing mice that will 1) extend the "eclipse" period in amputated mice and will 2) also provide progressive growth of mKSA (Tu 5) cells, tissue culture-propagated cells that have lost their oncogenic potential. This factor was found also in DBA/2 mice bearing L1210 ascites but not in ascitic fluid of several other ascitic tumors: FBL-3 and plasma cell neoplasms (syngeneic with mKSA). Fractionation studies are in progress to define this factor.

RNA Virus-Induced Neoplasms

d) The nature of the virus-determined cell surface antigens (CSAs) and of TSTA and of their interrelations and of the role of virion components P30, P15, GP 69/71 found on leukemic cells is a problem receiving much emphasis. A chromatographed fraction (F2 from G-150 Sephadex) was shown previously to provide activity in a ^{51}Cr-release cytotoxicity assay and to provide specific tumor rejection of FMR type leukemias. Through the use of a CF assay it was found that a specific component presumably not virion antigens existed in our F2 fraction that specifically lysed RBL-5 cells.

Since we now have the purified virion components P30 and GP 69/71 and antisera to those components (from Dr. S. Oroszlon, Flow Labs.) we are in the process of determining their role in the specific cytotoxic response employing our syngeneic antisera to membrane components and their role in tumor rejection, that is the presence of TSTA. We have found a) that P30 and GP70 are not responsible for cytotoxicity with our syngeneic antiserum and b) that they do not play a role in tumor rejection (using FBL-3 and RBL-5 in syngeneic B6 or semi-syngeneic CBF_1 mice. Solubilized antigen that has had virion components P30 and GP69/71 removed by passage through columns containing anti P30 and GP69/71 retain their capacity to immunize specifically as measured by tumor rejection.

Relation of H-2K and H-2D loci to tumor cell kill

e) Two groups of investigators have now reported that leukemic cell targets are lysed, as detected in a cell-mediated ^{51}Cr assay only when effector cells and target cells are syngeneic at the H-2K and/or H-2D loci. The most likely proposed mechanism is an alteration of the H-2 molecule or an interaction occurs between a viral and an H-2 molecule. The TAA in each case is not known nor are TSTA present in the systems investigated. More recently it has also been postulated that the H-2 type of cell used for immunization is also a decisive factor in the T-cell killers generated. We are presently investigating whether a) this phenomenon is characteristic only of the ^{51}Cr-release assay - by using an IuDR assay employing other than RNA virus-induced neoplasms and also investigating the *in vivo* significance of H-2 sharing employing matched and unmatched loci in the Winn assay.

Significance for Cancer Research:

Characterization of tumor antigens of the rejection type is a necessary prerequisite for understanding the mechanisms of immune surveillance, tumor inhibiton and facilitation. In addition the role of these membrane bound components in the actual process of carcinogenesis may be studied.

As a basis for any study of membrane bound antigens it is necessary to study the nature of histocompatibility antigens and their relationship to tumor antigens. Thus our emphasis is on parallel studies of H-2 antigens in order to provide a basis for understanding tumor antigens.

Objective 3; Approaches 4,5.

Publications:

Natori, T., Tanagaki, N., Pressman, D., Henriksen, O., Appella, E. and Law, L. W.: Two components of papain-solubilized H-2 antigen molecules. Immunogenetics (in press) 1976.

Law, L. W. and Appella, E.: Studies of Soluble Transplantation and Tumor Antigen. In Becker, F. F. (ed.): Cancer - A Comprehensive Treatise, Biology of Tumors Vol. IV: 135-157 (1975).

Law, L. W., Appella, E., Chang, K. S. S. and Henriksen, O.: Soluble tumor rejection antigens from membranes of virus-induced neoplastic cells. In Bucalossi, P., Veronese, U., and Cascinelli, N. (Eds.): Proc. of XI Internat. Cancer Congress, Florence, Italy, Amsterdam, Excerpta Medica, 1975, No. 349 Vol. 1. pp. 228-231.

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Law, L. W., Chang, K. S. S. and Ting, R. C.: Immunogenic properties of a non-producer malignant tumor induced by murine sarcoma virus. Proc. Soc. Exper. Biol. Med. (in press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 03222-08 LCBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Genetic and Biochemical Mechanisms Controlling Protein Synthesis in Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Mushinski	Senior Surgeon	LCBGY NCI
OTHER:	M. S. Marini	Visiting Fellow	LCBGY NCI
	M. Potter	Head, Immuno-chemistry	LCBGY NCI
	H. G. Coon	Chemist	LCBGY NCI
	E. P. Reddy	Project Director	Microbiological Assoc.
	I. B. Weinstein	Professor, Depts. of Medicine and Biochemistry	Columbia University
	D. Grunberger	Assoc. Prof. Dept. Biochemistry	Columbia University
	R. Salomon	Research Associate	Weizmann Inst. of Science

COOPERATING UNITS (if any)

Columbia University and Weizmann Institute of Science, Rehovot, Israel

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Immunochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	2.0	0.9

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the control mechanisms important in regulating protein synthesis in normal and malignant mammalian cells.

A. Transcription of and translation by tRNA is being studied by comparing isoaccepting tRNA chromatographic profiles of normal and tumorous tissue. Primarily rat and mouse liver tRNA is being compared with tRNA from rat and mouse tumors, particularly rat hepatomas and a carefully selected series of immunoglobulin secreting mouse plasma cell tumors. Of special interest is the origin, structure and function of a species of phenylalanyl-tRNA found only in certain rat tumors.

B. The synthesis and structure of IgA half molecules, mouse IgA myeloma proteins made up of one light chain disulfide bonded to one unusually short heavy chain, is also of major interest, particularly to determine if these molecules represent a normal IgA subclass or products of aberrant or incomplete synthesis at the DNA, RNA or protein synthetic level.

Methods Employed:

A. Transfer RNA from Mouse Myelomas, Rat Hepatomas and Other Tumors

The variations in isoaccepting tRNAs among tumors and normal tissues are being studied using: 1) reversed phase, high pressure liquid chromatography, 2) polynucleotide directed ribosome binding, 3) cloning in tissue culture, 4) cell free protein synthesis and 5) RNA sequencing techniques.

B. IgA Half Molecule Mouse Myeloma Proteins

These unusually small immunoglobulins are being studied immunochemically using: 1) homologous and heterologous antiserums in double gel diffusion and hemagglutination inhibition, 2) polyacrylamide gradient gel electrophoresis, 3) exo- and endopeptidase digestion, 4) cloning of mutagenized tissue cultures of IgA plasmacytomas, 5) isolation of messenger RNA, and 6) assay of normal mouse tissue extracts for immunological evidence of IgA_h.

Major Findings:

A. Transfer RNA

1. Phenylalanyl-tRNA from Various Tumors

We have described the first structural difference between an additional Phe-tRNA (Phe-$tRNA_1$) found in 3 rat hepatomas and that Phe-tRNA (Phe-$tRNA_2$) found in normal rat liver and tumors. This difference seems to involve the absence of a very highly modified, hydrophobic and fluorescent base called "Y" in Phe-$tRNA_1$ which is present in Phe-$tRNA_2$. Repeated study of chromatographic profiles of Phe-tRNA from a variety of rodent tumors has shown that Phe-$tRNA_1$ may really consist of two peaks which are so similar that they are hard to separate chromatographically. Both these peaks are seen in small amounts in the following rat tumors: Morris hepatomas 5123D, 7777, and 7800, Sarcoma LA-2, Walker mammary Ca. 256, Thyroid Cas. 1-2 and 1-6. Unusually large amounts of these human-specific Phe-tRNAs were found in the spontaneous rat plasmacytoma IR-162 and the Gross virus induced thymoma, French NTD. Negligible amounts of these tumor-associated molecules were found in Morris hepatoma 5123, spontaneous mammary Ca. 4M, and basophilic leukemia RBL-1. A variety of mouse plasmacytomas and spontaneous mouse hepatomas failed to show any evidence of tumor specific Phe-tRNA. Hepatoma 7777 and normal rat liver cells were established in tissue culture and at least one of the two parts of the tumor specific Phe-tRNA complex was found in the cultured hepatoma.

2. Mouse Plasmacytoma tRNA Comparisons

Several lines of BALB/c plasmacytomas were selected for tRNA studies because of the nature of the immunoglobulin they secreted. Two tumor lines secreting only kappa type light chains with identical amino acid sequences except at two positions in V_L, two lines secreting IgAs with identical light and heavy chains except for a single V_H residue difference and three IgA

lines with identical C_L, V_L and V_H but different C_H regions were studied. Two cloned lines of IgG producing tumors were compared with two non-producing daughter clones which spontaneously arose during tissue culture. So far the tRNAs for 11 of the 20 natural amino acids have been examined for these tumors as well as for another tumor grown with and without treatment with BUdR. Until the remainder of the 20 amino acids have been studied our conclusions must be tentative and preliminary, but so far no definite correlation between particular isoaccepting tRNAs and sequence differences in immunoglobulins secreted has emerged. There are striking differences in certain isoaccepting patterns, particularly Ser-tRNA and striking lack of differences among others, notably Gly-, Asp- and Leu-tRNAs.

B. IgA Half Molecules (IgA_h)

Direct comparison of the molecular size of six of these IgA_h myeloma proteins by polyacrylamide gradient gel electrophoresis shows that all six appear as L_1H_1 molecules of identical size, ca. 65,000 daltons, apparently the smallest immunoglobulins so far reported. In addition, all six have heavy chains of the same size, ca. 40,000 daltons, which would be the smallest immunoglobulin heavy chains known. Since proteolytic generation of these unusually small molecules from larger ones has been ruled out by our earlier intracellular kinetic studies, and since chance chain terminating mutations are unlikely to generate 6 molecules of identical size, we feel that these represent a second IgA subclass in mice. Homologous class-specific antiserums were prepared for IgA_h, and they detected immunologic evidence of IgA_h in normal BALB/c mouse gut contents. This is additional evidence that IgA_h is a normal IgA subclass in mice.

Significance:

A. The identification of a structural difference in Phe-$tRNA_1$ which is characteristic of nearly all rat tumors examined to date and absent in normal adult and fetal rat liver places the chromatographic tRNA differences seen among tumors on a firmer footing than heretofore. Since this particular structural alteration is immediately adjacent to the tRNA's anticodon, it is likely to significantly disturb or alter the fidelity of translation of messenger RNAs, with many potentially disruptive sequellae for cellular growth and metabolism. The critical questions now remaining are whether this Phe-$tRNA_1$ represents the transcription of a new tRNA gene only in tumors or results from an incomplete post-transcriptional modification of the normal Phe-$tRNA_2$, and if Phe-$tRNA_1$ functions normally or abnormally in protein synthesis.

B. We have evidence that IgA_h molecules constitute a second subclass of IgA not previously detected in normal mice. IgA_h-specific antiserum was able to detect the presence of these IgA_h determinants in normal mouse intestinal contents by precipitin reaction and hemagglutination inhibition. This recognition may inable us to learn more about the genetic origin, organization and regulation of expression of mouse immunoglobulin genes. Similarities with the two known subclasses of human IgA have been noted, but more study is required to prove how analagous the two subclasses of mouse and human IgAs are.

Proposed Course:

A. We have expended nearly 8 months in growing up large amounts of hepatoma 7777 in rats and preparing tRNA from these tumors. Our collaborators are further purifying Phe-tRNA$_1$ and Phe-tRNA$_2$ for detailed sequence analysis which is designed to tell us if these two tRNAs come from separate genes or represent different degrees of post-transcriptional modification. Further studies with the two purified, separated Phe-tRNAs will examine the coding characteristics of the Phe-tRNAs and how well they translate natural and artificial messengers in cell-free protein synthesis. Attempts to transform normal hepatocytes in tissue culture with carcinogens and BUdR will be accompanied by isolation of tRNA to see if Phe-tRNA$_1$ appears concurrently with transformation.

We plan to complete the chromatographic comparison of selected plasmacytoma tRNAs for all 20 amino acids. At that time we will assess how much isoaccepting tRNAs change when a mutation in a structural or regulating gene occurs in tumor lines and how closely the tRNAs reflect the nature of the major secretory protein product of plasmacytomas. In this way we hope to learn how tRNA expression is regulated, and what role tRNA could play in transformation and in mutational events.

B. Further characterization of the IgA$_h$ subclass of mouse immunoglobulins will be pursued primarily along the following lines: 1) compare sensitivity to proteases to see how well such molecules can survive intact in gut environment, 2) subjecting polymerizing IgA (IgA$_p$) myeloma proteins to controlled proteolysis to rule out proteolytic production of IgA$_p$ fragments in the gut which might mimic IgA$_h$ antigenically, 3) develop a more sensitive radioimmunoassay to detect IgA$_h$ elsewhere in normal mice, 4) test the native IgA$_h$ for antigen binding activity, and 5) to search for IgA$_h$ in NZB myeloma proteins to develop reagents which could test for genetic distribution of BALB/c vs. NZB allotypic examples of IgA$_h$.

Relationship:

Objective - 3

Approach - 1

Publications:

Marini, M. and Mushinski, J. F.: Aminoacyl-tRNA profiles related to the proteins secreted by mouse plasmacytomas. Accepted by Proceedings of American Association for Cancer Research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 03229-08 LCBGY

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

I. Chemical structure of immunoglobulin molecules and allotypes
II. Purification and biochemical characterization of soluble murine H-2 antigens, human HL-A antigens, and tumor specific antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ettore Appella	Medical Officer (Res.)	LCBGY	NCI
OTHER:	Ole Henriksen	Sr. Staff Fellow	LCBGY	NCI
	Michael Rogers	Sr. Staff Fellow	LCBGY	NCI
	Maria Prat	Visiting Fellow	LCBGY	NCI
	Takashi Natori	Expert	LCBGY	NCI
	John Inman	Biochemist	LI	NIAID
	Rose Mage	Immunochemist	LI	NIAID
	N. Tanigaki	Biochemist	Rosewell Park	
	O. Roholt	Biochemist	Rosewell Park	

COOPERATING UNITS (if any)

Roswell Park Memorial Institute, Buffalo, New York
Laboratory of Immunology, NIAID

LAB/BRANCH

Laboratory of Cell Biology

SECTION Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	4.50	0.75

SUMMARY OF WORK (200 words or less - underline keywords)

Chemical and Structural Studies of Antibodies and Histocompatibility Antigens. It is the purpose of this project to study the chemistry and structure of antibodies and mouse and human histocompatibility antigens. Antibodies of different specificities and allotype are purified and affinity labeled of the binding site. The chemical sequence of both the light and heavy chains is obtained in order to gain some insight into the nature of antibody specificity and the generation of diversity. Improvements in the isolation methods of histocompatibility antigens are currently being pursued. The characterization of the chemical structure of these proteins is being approached by micro methods and the relationship of the amino acid sequence to immunoglobulin structure is being analyzed.

Project Description:

I. Rabbit Immunoglobulins:

Studies of the structural basis of antibody specificity have involved labeling the antibody combining site and localizing the label within the primary structure of the antibody molecule. The results have all shown that the labels are found within the hypervariable sequences in the variable region of the heavy and/or light chains. Using the paired-iodination technique, we have extended our studies to a second rabbit anti-p-azobenzoate antibody and a tyrosyl residue at position 30 in the light chain has been identified as being in the binding site. These studies together with data reported previously for the light chain of an antibody directed toward the p-azobenzoate determinant demonstrate that both the first and third hypervariable regions in the light chains may contribute to the binding site structure. Thus, there are more than one set of combining site sequences complimentary for a given determinant. The antigenic determinants of rabbit IgG include isotypic specificities and allotypic specificities. The heavy chain allotypes a1, a2 and a3, behave as if controlled by three allelic genes at an autosomal locus. Chemical analyses have established that there are approximately 16 residues in the variable regions of heavy chains which correlate with a locus specificities. Direct proof for the localization of an antigenic determinant, however, can be obtained from isolation of peptides carrying a allotypic specificities. An immunopeptide bearing a3 allotypic determinants has been isolated from the heavy chain of an a3 homozygous rabbit. Its molecular weight was found to be 6,000. This peptide is composed of two polypeptide chains linked by a disulfide bond. It covers positions 17-34 and 64-94 of the variable region. Most of these residues are fully exposed on the surface and are far removed from the antibody combining site. Further structural studies are in progress and may help to define the nature of the variable gene markers.

II. Histocompatibility Antigens.

Papain-solubilized HL-A antigens have been shown to contain two polypeptide fragments: β_2-microglobulin with a molecular weight of 12,000 and a larger fragment with a molecular weight of about 34,000. The large fragments isolated from two HL-A preparations carrying different specificities appeared homogeneous both by immunoelectrophoresis and SDS-acrylamide electrophoresis. The carbohydrate content of the large fragment was 12.9% making the molecular weight of the polypeptide chain of the large fragment approximately 30,000. Small but significant differences have been found in the amino acid compositions and tryptic peptide maps of the large fragment containing different specificities. Both HL-A antigen preparations contained the same NH_2-terminal, glycine. Partial amino acid sequence has been obtained for the first 22 residues. With the exception of one possible difference in position 9, the sequences of the two HL-A preparations are identical. This supports earlier suppositions that the two HL-A loci are homologous to one another and must have come from a common ancestral gene. For the mouse H-2, various cultured cell lines, liver and spleen cells have now been chosen as the source of material.

A crude membrane preparation is prepared by differential centrifugation according to established procedures. The membranes are digested by a limited papain digestion which solubilizes H-2. The solubilized H-2 is further purified by gel filtration, affinity chromatography and isoelectric focusing. The purified H-2 is shown to contain two components after SDS-gel electrophoresis; one with a molecular weight of approximately 35,000 and a small component with a molecular weight of approximately 12,000. These observations are in agreement with reports from other laboratories in which trace amounts of H-2 have been prepared. Chemical characterization of the purified large fragments is now in progress. Detergent solubilized preparations are also being purified. Membranes from $H\text{-}2^b$ haplotypes have been carried through a number of steps. This material as judged by SDS-PAGE and immunoprecipitation of radiolabeled antigens is now about 20% pure.

A highly purified preparation of mouse β_2-microglobulin has been obtained from sodium thiocyanate extracts of liver cell membranes. The first 40 residues have been sequenced. The mouse protein differs only at 9 positions from human β_2-microglobulins. The sequence also has homology to the constant regions of mouse γG2a, most closely to the C_H3 region. These data support the conclusion that the mouse protein is the mouse homologue of human β_2-microglobulin. Mouse β_2-microglobulin in serum has been found to be associated with a substance of approximately 40,000 molecular weight lacking H-2 alloantigenic specificities. The nature of this protein remains at this moment unknown. The possibility that other specificities for example, of the Ia type are indeed carried by this protein, are being actively investigated. This situation resembles the allogeneic effector factor, where both Ia and β_2-microglobulin antigenic determinants have been shown to be present.

Another aspect of our work is the analysis of the H-2 antigens on tumor cells. A monospecific antiserum which will detect $H\text{-}2.33$ ($H\text{-}2^b$) was found to be cytotoxic for DMLM 1678 ascites tumor cells of the $H\text{-}2^s$ haplotype. The H-2 molecules or variants seem to be involved in this reaction and not viral proteins. The importance of this observation is obvious, in view of several suggestions that H-2 antigens are important in the lysis of syngeneic cells.

III. Tumor Specific Transplantation Antigens (TSTA).

SV40 induced TSTA has been solubilized by limited papain digestion. Quantitative studies have been carried out on the immunogenicity of different fractions from dose-response curves relating tumor size and the amount of protein used for immunization. It was found that only 2-3% of the TSTA-activity of the membrane preparation was recovered in the papain-solubilized fraction. Fractionation of the solubilized material by chromatography on DEAE-cellulose, Sephadex-G150 and concanavalin-A-Sepharose columns did not concentrate the immunogenic activity. These results are in contrast to the purification of H-2 antigens using the same methods, and point to a higher susceptibility of the TSTA to papain digestion. In examining immunity to Friend virus induced leukemia, the role of the major viral structural proteins p30 and gp70 has been assessed. Highly purified p30 has no effect when used to immunize animals with microgram quantities against subsequent challenge with FBL-3 ascites tumor or RBL-5 subcutaneous tumor. Purified gp70 shows

only weak protection against the RBL-5 subcutaneous tumor. Neither protein significantly inhibits cell-mediated cytotoxicity of immune lymphocytes toward FBL-3 tumor cells, or humoral cytotoxicity of syngeneic hyperimmune serum toward RBL-5 tumor cells. Thus, these two proteins do not appear to function as tumor specific transplantation antigens in this system.

Publications:

Law, L. W., Appella, E., Chang, K. S. S. and Henriksen, O.: Soluble tumor rejection antigens from membranes of virus-induced neoplastic cells. In Bucalossi, P., Veronese, U., and Cascinelli, N. (Eds.): Proc. of XI Internat. Cancer Congress, Florence, Italy. Amsterdam, Excerpta Medica, 1975, No. 349 Vol. 1. pp. 228-231.

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Friedenson, B., Appella, E., Roholt, O., Wright, J. D., Tissot, R., Cohen, C. and Pressman, D.: Location of light chain tyrosine peptides from the combining site of an anti-p-azobenzoate antibody. Eur. J. Immunol., (in press) 1976.

Maurer, B. A., Dean, J. H., McCoy, J. L., Appella, E., and Law, L. W.: Cell-mediated immunity in mice against papain-solubilized histocompatibility and tumor specific antigens by a macrophage migration inhibition microassay. J. Nat. Cancer Inst. (Accepted for publication).

Law, L. W., and Appella, E.: Biological and biochemical properties of solubilized histocompatibility-2 (H-2) alloantigens. In Inman, F. P. (Ed.), Contemporary Topics in Molecular Immunology, Plenum Press, N. Y. (Accepted for publication).

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Carta-Sorcini, M., Ansari, A. A., Mage, R. G. and Appella, E.: Studies on the structural localization of rabbit H chain allotypic determinants controlled by the a locus: Purification and properties of an immunopeptide bearing a3 allotypic determinant(s). J. Biol. Chem., (in press) 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 04833-08 LCBGY

PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biological Studies of Various Normal and Virus-Infected Malignant Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. A. Wivel	Head, Ultrastructural Biol.	LCBGY NCI
OTHER:	S. S. Yang	Chemist	LCBGY NCI
	H. L. Malech	Res. Assoc.	LCBGY NCI

COOPERATING UNITS (if any)
M. Potter LCBGY NCI
P. Pitha Johns Hopkins University School of Medicine

LAB/BRANCH Laboratory of Cell Biology

SECTION Ultrastructural Biology Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.50	2.50	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the primary purpose of this project to study some of the pertinent factors which influence cell differentiation and malignant transformation, using techniques and approaches which range from the microscopic to the molecular level. Particular emphasis is given to those systems in which murine RNA tumor viruses or their precursors may be the transforming agent. A variety of mouse model systems are used, including plasma cell tumors, mammary tumors, and neuroblastomas. Current projects include: 1) characterization of the properties of intracisternal A particles; 2) relationship between type C viruses and solid tumors other than sarcomas; 3) correlation of cell ultrastructure with immunoglobulin secretion in Abelson virus-induced plasma cell tumors and lymphosarcomas; 4) effects of interferon on assembly of murine oncornaviruses.

PHS-6040
(12-75)

Major Findings:

1. Intracisternal A particles. It has been previously established that intracisternal A particles (IAP) have several biochemical characteristics known to be associated with murine oncornaviruses, namely high molecular weight RNA and reverse transcriptase. It has also been shown that IAP can be detected in the two-cell embryo, suggesting that vertical transmission can occur. However, until now there has been no evidence that IAP can be transmitted horizontally; demonstration of this capability would lend credence to the concept that IAP have infectious properties. Through the use of techniques for somatic cell hybridization, we have been able to transfer IAP from enucleated IAP-positive cells (cytoplasts) to IAP-negative cells by fusing them in the presence of inactivated Sendai virus. Such cytoplast-whole cell hybrids are designated as cybrids. By using mutant markers the identification of both parent lines and the resulting cybrids could be achieved. IAP-positive BALB/c mammary tumor tissue culture cells (MT) were mutagenized and selected for resistance to chloramphenicol (CAP) and ouabain while 3T3-4EF cells (IAP-negative) were selected for resistance to BUdR. As a preliminary experiment it was determined that the CAP resistance could be transferred in the cytoplasm by fusing the mammary tumor cytoplasts to sensitive cells. MT cytoplasts were then fused to 3T3-4EF cells and selected in CAP and BUdR. The resulting cybrid clones were resistant to CAP and BUdR and sensitive to ouabain, and had marker chromosomes and the phenotype of 3T3-4EF cells. 80% of the clones showed continued IAP production over some 60-90 cell generations. These results suggest that the genetic information coding for IAP can be transferred in the cytoplasm and thus horizontal transmission can occur under appropriate conditions. The cybrid cell lines represent an important development for studying the mode of replication of IAP, i.e., they can be used to answer the following questions: Does fusion allow for establishing an infection with IAP or does it activate an IAP genome already in the recipient cell? Appropriate nucleic acid hybridization experiments should clarify this situation. If the parent 3T3-4EF line does not contain IAP genes and the cybrid does, then it is probable that infection has taken place. However, if the 3T3-4EF cells do contain IAP genes, then fusion may have served to activate a preexisting genome. Since it is now possible to delineate this potential for infectivity, a major question regarding the ultimate identity of IAP as viruses can be answered.

2. Neuroblastoma cells and C-type virus expression. Although there have been unconfirmed reports that C-type viruses can cause neuroblastomas in strain A mice, there is no unequivocal evidence that such viruses cause any neoplastic lesions other than leukemias and sarcomas. In an attempt to clarify some of the relationships between MuLV and neuroblastoma, we studied three tissue culture clones derived from the C1300 tumor. None of the clones released endogenous ecotropic virus spontaneously, but one did release xenotropic virus. Two of the clones could be induced to release virus when treated with IUdR and dexamethasone, but one clone, N-18 did not release either ecotropic or xenotropic virus under a variety of induction conditions. All three clones showed evidence of gs-1 antigen as measured by immunofluorescence. Although clone N-18 did not release virus, these tissue culture cells were still capable of

producing tumors in the mouse. Thus the ability to control virus production was independent of the oncogenic potential. Clone N-18 provides an excellent in vitro system for studying factors that regulate C particle gene expression, particularly since these cells are derived from a mouse strain with low leukemia incidence.

3. Ultrastructure of plasma cell tumors and lymphosarcomas. It is now apparent that Abelson MuLV can act as a co-carcinogenic agent in concert with Pristane to rapidly induce plasma cell tumors in BALB/c mice. However, some of the tumors develop before differentiation into plasma cells is complete, that is, there is a whole spectrum of neoplasms ranging from lymphosarcomas to intermediate forms to classical myelomas. We have begun to better characterize this system by comparing the tumor ultrastructure with the ability to secrete immunoglobulin and produce IA antigen. A representative survey of several tumors indicates that the secretory products are found only in those cells which can be classified as plasma cells or as lymphocytes with a fairly extensive rough endoplasmic reticulum and a prominent Golgi complex. Those tumors containing only small lymphocytes with free ribosomes appear to be unable to secrete either immunoglobulin or IA antigen.

4. Interferon treatment of murine oncornaviruses. In a collaborative study with Dr. Paula Pitha, we have been studying the effects of interferon on in vitro AKR virus infection. Results of these studies indicate that the presence of interferon causes a block in the assembly of the virus, with an arrest at the budding stage. On a quantitative and statistically significant basis, treated cells show many more budding particles than controls and such buds are not released from the plasma membrane. This lack of virus release accounts for the lower titer seen in the tissue culture medium from interferon-treated cells. Further studies will be directed toward determining some of the mechanisms which cause this effect on the replicative cycle.

Relationship of Research to National Cancer Plan:

Project Area - 10

Approach Elements - 2

Approach - 4

Publications:

Wivel, N. A., Smith, G. H., and Ozer, H. L.: Comparison of intracytoplasmic A particles and intracisternal A particles. Int. J. of Cancer 16: 240-248, 1975.

Malech, H. L. and Wivel, N. A.: Properties of murine intracisternal A particles. Electron microscopic appearance after critical point drying and platinum shadowing. Virology 69: 802-809, 1976.

Yang, S. S. and Wivel, N. A.: Physicochemical analysis of the deoxyribonucleic acid product of murine intracisternal A particle RNA-dependent DNA polymerase. Biochim. Biophys. Acta (1976) 407, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 04834-01 LCBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Viral gene expression in normal tissues and in Virus- or Chemically Induced Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. S. Yang Chemist LCBGY NCI

COOPERATING UNITS (if any)
N. A. Wivel Head, Ultrastructural Biology Section LCBGY NCI
E.I.B. Thompson Head, Biochemistry of Gene Expression Section LB NCI

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Ultrastructural Biology

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	.50	1.00

SUMMARY OF WORK (200 words or less - underline keywords)

The major thrust of this study is to elucidate the basis of neoplastic transformation of normal tissues. Two experimental model systems were used in this project: (1) a rat hepatoma induced by the chemical carcinogen N,N-2,7-fluorenylene bis-2,2,2-trifluoroacetamide, which has been adapted to tissue culture (HTC cells); and (2) two intracisternal A particle (IAP) containing neoplasms, a plasma cell tumor (MOPC-104E) and differentiated neuroblastoma, also adapted to tissue culture (cell lines N-4, N-18, and NB-2A). Multi-disciplinary approaches were required in this investigation. They involve tissue culture, biochemical and biophysical analyses, cytogenetics, immunology and tumor biology. Areas with special emphasis of research effort are: (1) the induction and/or rescue of viral (C-type) genome in chemically induced hepatoma; (2) the causal or temporal involvement of this C-type virus in the carcinogenesis of rat hepatoma; (3) the nature of virus-chemical interaction during the process of co-carcinogenesis; (4) cell regulation of viral gene expression on the genetic level; (5) virus infection and the development of chromosome abnormalities during the process of neoplastic transformation, and (6) integration of viral genome and its localization on host cell chromosome(s).

Major Findings:

I. Genome Rescue and Characterizations of a Rat Hepatoma Helper Virus Pseudotype Kirsten Sarcoma Virus.

Although much work has been done in the areas of tumor biology the basis of how tissues come to lose their normal cellular control mehcanism over metabolism and develop into neoplastic state remains to be elucidated. Currently the major effort in this laboratory is concentrated toward a better understanding of neoplastic transformation by adopting as an experimental model system the chemically induced rat hepatoma, which has been adapted to in vitro growth in tissue culture, called HTC cells. HTC cells are heteroploid rat tumor cells established from an ascitic form of the rat solid hepatoma 7288c originally induced in 1962 by the chemical carcinogen, N,N-2,7-fluorenylene-bis-2,2,2-trifluoroacetamide (Morris, H.P. (1965) Advances in Cancer Research 9, 262-266). Biochemical properties, principally those involved in corticosteroid responses and some aspects of cytogenetics of HTC cells have been well studied (Yang et al. (1974) Endocrinology 94, 254-261; Lippman, Yang and Thompson (1974) Endocrinology 94, 262-266). These included the induction of tyrosine aminotransferase, altered cell surface characteristics, and specific increases in one iso-accepting form of phenylalanyl transfer RNA as a result of dexamethasone phosphate (DXP) treatment of HTC cells. These facts, together with the augmentation of virus induction in mouse systems by DXP, led us to attempt similar experiments in HTC cells. However repeated attempts of virus induction in a few HTC lines including HTC-H1, treated with either 5-iodo-deoxyuridine (IUdR) or 5-bromo-deoxyuridine (BUdR) at varying dosages optimal for induction of C-type viruses in the mouse systems, with DXP, failed to elicit increased level of reverse transcriptase activity as compared to untreated HTC cells. Neither was the release of infectious C-type virus observed in these chemically treated HTC cells when conditioned media from these cultures were assayed for cell transformation activity. Recently extensive efforts in genome rescue experiments resulted in the isolation of a rat hepatoma virus pseudotype Kirsten sarcoma virus, KiMSV(RHHV), when HTC-H1 cells were co-cultivated with Kirsten sarcoma virus transformed non-producer rat kidney cells (K-NRK). KiMSV(RHHV) is now cultured at a high titer (10^4 focus forming units/ml of medium) in vitro. Biological, morphological, biochemical and immunological characteristics of KiMSV(RHHV) are currently under investigation. It has a C-type virus ultrastructure. It is strictly rat tropic in host range. The helper virus, RHHV, originates from the hepatoma cell as suggested by the mode of its rescue and by its host specificity. Nucleic acid hybridization analysis is now carried out to further substantiate this point. Results obtained by indirect fluorescent-antibody assays and neutralization bioassays indicate that KiMSV(RHHV) exhibits a major rat leukemia virus (RaLV) specific antigen (GP 70, glycoprotein of 70,000 mol. wt.) and an inter-mammalian group specific gs-3 (P30, 30,000 mol. wt. protein) antigen. The major viral protein associated with KiMSV(RHHV), when the virus was labelled in either ^{35}S-methionine or ^{3}H-amino acids and then analyzed by polyacrylamide gel electrophoresis, is a 30,000 mol. wt. protein. A very minor radioactive band can also be consistently detected at the 70,000 mol. wt. area; whether this 70,000 mol. wt. material represents a membrane glycoprotein

that accounts for the neutralization specificity of RaLV or enzymatic components of the virus remains to be resolved. We are now attempting to radioactively label KiMSV(RHHV) at a higher specificity so that other minor protein fractions may also be detectable. KiMSV(RHHV) sediments isopycnically in a linear sucrose gradient at 1.145-1.155 g/cm^3, less dense than the known mouse C-type tumor viruses. It also possesses the complements of RNA and reverse transcriptase. Its possible tumorigenic and immunogenic effects in vivo are under investigation in a long-termed project. We have observed so far that when KiMSV(RHHV) transformed Fisher rat embryonic cells were inoculated into syngeneic new-born or young adult rats, not only was there rapid growth of a capsulated tumor at the site of inoculation, but metastases were also seen primarily to the liver.

II. Intracisternal A Particle Containing Neuroblastoma Cell Lines and their use in Studying Cell Regulation of Viral Gene Induction and Expression on the Genetic Level.

This project is a co-operative effort with Dr. N. A. Wivel, Head of the Ultrastrastructural Biology Section. Details of the major findings of this study is included in this Annual Report under project number Z01 CBD 04833-08 LCBGY. Briefly, three tissue culture clones of neuroblastoma cells derived from the C-1300 tumor in strain A/J mice were found to contain type-C virus specific gs antigen. None of the clones spontaneously released mouse-tropic type-C viruses although one clone, N-4, spontaneously released a xenotropic virus Two clones, NB-2A and N-4, could be induced by treatment with 5-iodo-deoxy-uridine and dexamethasone phosphate to produce B-tropic type-C virus, but clone N-18 failed to respond to various virus induction conditions. Karyotype analysis did not reveal specific chromosome deletions in any of the neuroblastoma clones. The varying degrees of C-type virus induction and expression among these neuroblastoma clones can best be explained on the basis of evolutionary divergence in regulatory gene control of the neuroblastoma clones in their response to virus induction and expression.

III. Physicochemical Properties of the Endogenous DNA Transcript of Intracisternal A Particle Reverse Transcriptase.

In an on-going project with Dr. N. A. Wivel the endogenous DNA product of IAP reverse transcriptase was analyzed in detail by various physicochemical methods. ascent DNA transcript of IAP reverse transcriptase appeared to be covalently linked to an RNA primer. Fidelity of transcription is evident since the DNA transcript hybridized specifically back to the 35-70S RNA of IAP but not with heterologous RNAs. This DNA transcript has an approximated M.W. of 145,000, estimating 350 polynucleotides and base ratios with an excess of dGMP.

Relationship of Research to National Cancer Plan:

Project Area - 10

Approach Elements - 2

Approach - 4

Publications:

Yang, S. S., Chan, C. and Thompson, E. B.: Rescue of a rat tropic rat hepatoma virus pseudotype Kirsten sarcoma virus by co-cultivation of hepatoma tissue culture cells with K-NRK cells. J. Gen. Virol. (1976) 31, in press.

Yang, S. S. and Wivel, N. A.: Physicochemical analysis of the deoxyribonucleic acid product of murine intracisternal A particle RNA-dependent DNA polymerase. Biochimical et Biophysical Acta (1976) 407, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05526-08 LCBGY

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cell Surface Changes in Spontaneously or by SV40 Transformed Mouse Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. T. Mora	Head,Macromolecular Biology Section	LCBGY DCBD NCI
OTHER:	J. Coll	Visiting Fellow	LCBGY DCBD NCI
	C. Chang	Visiting Fellow	LCBGY DCBD NCI
	D. Huang	NIH Postdoctoral Fellow	LCBGY DCBD NCI
	J. Küster	Visiting Fellow	LCBGY DCBD NCI
	D. Winterbourne	Visiting Fellow	LCBGY DCBD NCI

COOPERATING UNITS (if any)
R. G. Martin, LMB, NIAMDD
P. W. Kent, University of Durham, Glycoprotein Research Unit, Durham, England

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Macromolecular Biology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	4.0	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the cell surface, how it may change in transformation to malignancy, either spontaneously, or as induced by SV40 virus. Topics of present interest are 1) Changes in protein and glycoprotein components, and correlation with cell growth properties in tissue culture and in vivo in the syngeneic and in the nude mice; 2) Controls of expression and methods of purification of surface antigens and tumor specific transplantation antigens; 3) Selection of phenotypic and genotypic revertant cells which lose SV40.

Project Description:

Objectives: To elucidate certain biochemical and biological changes on the cell surface which occur during transformation to malignancy in mouse cells.

Methods Employed: Labelling glycoproteins, proteins and glycolipids, also of nucleic acids with radioactive precursors during cell growth in culture. Novel fractionation techniques for isolation of (labelled) cell surface membranes. Special chemical isolation and characterization procedures in thin-layer and column chromatography, gel electrophoresis, and in autoradiography of gel slabs. Analytical and preparative ultracentrifuge studies. Enzymology techniques pertinent to intermediary metabolism of carbohydrates. Serum mediated cytotoxicity assays as measured by release of radioactivity from lysed cells. Cell growth dynamics in culture, and also in syngeneic and in nude mouse.

Major Findings:

I. Changes in cell membrane components in exponentially growing mouse fibroblast cells.

A family of mouse fibroblast cell lines including normal, spontaneously and SV40 virus transformed lines, and clones and subclones thereof, were compared in exponential phase of growth in protein constitution of their cell membranes (work with Drs. Coll and Luborsky). By monitoring for specific (enzymatically removable) cell surface components, such as labelled sialic acid, we developed a method (Luborsky) to obtain preparations from these cells enriched in cell surface membrane. We observed one (glyco-) protein component (apparent mol. weight 180,000 as characterized by acrylamide SDS gel electrophoresis and autoradiography) to be reduced or absent in an SV40 virus transformed mass cell line when compared with the normal mass cell line. No such compositional difference was observed using protein staining only in a spontaneously transformed tumorigenic clonal derivative cell line, or in sister subclones of such derivative cell line with or without SV40 virus infection. However, in metabolic labelling experiments with ^{14}C mixed amino acids a consistent decrease also was demonstrated in the biosynthesis of the same (glyco-)protein in the SV40 virus infected subclones, as compared to uninfected sister subclones.

In the cell lines investigated no correlation was discernable between the membrane (glyco-)protein constituent in the exponentially growing cells and tumorigenicity or saturation density. Apparently the cells ability to grow to high density in monolayers in attachment, and also the tumorigenicity is not a simple correlate of the protein change in the membranes, as detectable by the techniques employed. However, the ability of the SV40 transformed cells to grow well without attachment in viscous suspension in methocell relates to the observed membrane differences, that is to the reduced amount of the 180,000 molecular weight (glyco-)protein constituent.

Previous studies by others showed differences in high molecular weight proteins in confluent or post confluent cell layers between untransformed and virally transformed fibroblastic murine cell lines, or cell lines transformed by temperature sensitive viruses grown at the permissive temperature vs. those grown at non-permissive temperature. Such differences are now known to be attributed to the fact that untransformed cells became arrested in a G_1 (or G_o) phase of growth when reaching confluence in a monolayer, but the (virally) transformed cells continued to divide. Our observation of differences in protein patterns and in metabolic labelling in exponentially growing cells pertain to the presence and functioning of SV40 genes (as expressed by production of T and tumor specific surface antigens), and to our knowledge it is the first which directly correlates membrane protein changes with SV40 transformation and not with indirect effects of serum and hormonal factors in the different stages of growth. There were no previous studies at all on the membranes of spontaneously transformed tumorigenic cells to contrast them with normal or virally transformed related cells.

The cell surface membrane purification techniques were greatly helped by collaborative studies on biochemical control of glycoprotein biosynthesis pertaining to cell surface membranes, carried out on our cells at the Glycoprotein Research Unit by Drs. P. W. Kent and D. Winterbourne, and now in part continued in our laboratory by Dr. Winterbourne. Direct results of such studies also led to recognition that there are further differences in mucopolysaccharides, most clearly seen between the spontaneously transformed and SV40 transformed cell lines. Glucosamine labelled, papain digest glycoproteins after ion exchange chromatography showed that differences also occur in hyaluronic acid, heparin sulphate and chondroitin sulphate. New SJL mouse skin fibroblast cell lines were developed to study by independent techniques the differences in the biosynthesis of these and other related mucopolysaccharides (D. Huang).

Cells were also compared by an external chemical labelling technique of proteins. The technique was developed in collaboration with Drs. Pancake and Luborsky (see Project No. Z01 CBD 05544-07 LCBGY). It included reacting the accessible external protein NH_2 groups on the cell surface with pyridoxal phosphate, followed by reduction of the resulting Schiff base with Na B^3H_4. The results have indicated small differences in cell membrane constituents between the various cell lines which were compatible with the definite and well characterized difference detected by metabolic labelling in the 180,000 mol. weight (glyco-)protein (see above).

II. Controls of expression and methods of purification of surface antigens and tumor specific transplantation antigens.

A. Detergent solubilization and partial purification of tumor surface and transplantation antigens from SV40 transformed AL/N mouse cells (with C. Chang, S. W. Luborsky, see Project No. Z01 CBD 05544-07 LCBGY, and S. J. Pancake).

Transformation of mammalian cells by SV40 is accompanied by virus specific antigenic surface changes which can be detected in vitro by serological tests as tumor specific surface antigens (TSSA) and in vivo as tumor specific transplantation antigens (TSTA). SV40 transformed AL/N cells and other SV40 transformed cells from different strains of mice were solubilized with 0.5% Triton x-100 in TBS. This solubilized extract was found to be active both in inhibition of antibody-mediated cytotoxicity and in immunization of mice against subsequent syngeneic SV40 transformed tumor cell challenge. Stepwise ammonium sulfate (AS) precipitation of the extract provided 3 fractions. TSSA activity was detectable in the 30-50% AS precipitate; TSTA activity was found in both 0-30% AS fractions. Further fractionation of the 30-50% AS precipitate by Sephadex G-150 chromatography showed that TSSA can be separated into two fractions - one, eluting at the region of apparent mol. wt. of 45,000, and the other near the void volume. Both of these fractions also were found to contain TSTA. Similar treatments of spontaneously transformed AL/N cells and of normal AL/N mouse brain cells did not show activity either in vivo or in vitro. Thus both TSSA and TSTA can be solubilized using detergent, and then further purified. This permits us to study the correlation of in vitro cytotoxicity and in vivo transplantation-rejection tests. Other characterization techniques including ion exchange chromatography (DEAE and phosphocellular columns) and ultracentrifugation in density gradients are being used for further characterization and separation of the various antigens.

B. Study on expression of the various antigens with temperature sensitive SV40 mutants (C. Chang in collaboration with J. Anderson and R. G. Martin).

With the availability of tsA mutant SV40 virus, and of cells transformed by such virus, it became possible to correlate tissue culture growth property of the cells with the expression of the various SV40 antigens at the permissive (33°) and non-permissive (40.5°) temperatures. By a modified system based on our previous cytotoxicity assay (Pancake and Mora), we were able to employ Balb/c mice to produce antiserum, and after absorbing these antiserum by various cells we could determine the SV40 specific surface antigens on such cells by determining the residual cytotoxicity on ^{51}Cr labelled SV AL/N target cells. The antiserum was shown to be specific in that it was absorbed only by SV40 transformed cells, but not by normal, by spontaneously transformed, or by methylcholantrane induced tumorigenic cells. The wild type SV40 transformed Chinese hamster lung cells, or representative A-mutant transformant such cells grown at the permissive temperatures and at non-permissive temperatures absorbed out the antiserum well, at about equal cell number. Similar observations were made on normal mouse (Swiss embryo) brain cells, transformed by wild or tsA mutants and grown at the two temperatures. Soluble membrane extracts obtained by the detergent technique (see above) were tested for ability to prevent an SV40 transformed Balb/c tumor to take as ascites in the Balb/c mouse. Significant protections were obtained by extracts from wild type transformed cells, or by cells transformed by the tsA mutant grown at both permissive and non-permissive temperatures.

The expression of these surface antigens were consistent also with the expression of T antigen at both temperatures by the particular tsA mutant (Martin and Anderson). Thus although these mutant cells lose their ability to grow in low serum or to form colonies on a normal cell layer or on soft agar under non-permissive temperature, they still contain tumor surface and transplantation antigens, and also T antigen. Currently other tsA transformed rat cells (from Osborne and Weber) which lose T antigen at the non-permissive temperature, are under study for modulation of surface and transplantation antigens. The solubilization and fraction techniques referred to above are being used to study the relationships between T antigens and TSSA and TSTA also in other cell systems (Dr. Chang).

III. Studies on the correlation between tumorigenicity and the immunologic rejection of spontaneously transformed cells further transformed by SV40. Selection of SV40 revertant (tumor) cells (with Dr. Chang).

It is well known that cells kept for prolonged period in tissue culture often become tumorigenic spontaneously. One such cell line from AL/N strain mouse embryo, the T AL/N mass cell line, was the subject of extensive biologic and biochemical studies in this laboratory. A noteworthy feature of this T AL/N cell line is its very high malignancy: less than 10^2 cells cause rapidly progressing and lethal fibrosarcomas in the syngeneic AL/N mouse. On the other hand when the same normal mouse embryo mass cell line from which the T AL/N cell line arose was infected and transformed by SV40 virus, the resulting SV AL/N line did not cause tumors at 10^8 cells/mouse even after very prolonged cultivation, apparently because of SV40 specific (transplantation) antigen(s) which were detected on their surface.

It became of interest to determine what will be the effect of SV40 transformation on the highly tumorigenic T AL/N cells. Transformation by SV40 is a highly inefficient process. To avoid selection of those T AL/N cells which are susceptible to transformation by SV40 and coincidentally may have innate or inducible difference in their tumorigenic potential, and to allow the study of the direct effect of the SV40 gene(s) insertion into a cell genome, we chose a cloned derivative cell line of T AL/N designated T AL/N clone 3. The T AL/N clone 3 cells were exposed to SV40 virus under conditions when somewhat below half of the cells were transformed as detected by SV40 T antigen and then the cells were recloned.

Both T antigen positive and negative subclones were obtained. The SV40 transformed T antigen positive clones ahd greatly reduced tumorigenicity in the immunologically competent syngeneic mouse, coincident with SV40 specific cell surface antigens. These cells had a high growth potential in the thymus-deficient nude mouse, and also in tissue culture when grown in suspension in a semi-solid medium. The T and surface antigen negative clones remained highly tumorigenic in the syngeneic mouse, and grew poorly in the semi-solid medium. This showed that transformation by SV40 of spontaneously tumorigenic cells can lead to immunological changes, resulting in the loss of tumorigenicity; also that the ability of cells to grow without anchorage in

semi-solid medium is not a simple correlate of tumorigenicity in the syngeneic host, as recently claimed by others to be in the nude mouse.

When the T antigen positive cloned cells were injected into syngeneic mice (10^4 cells) T antigen negative tumors were obtained. Cell lines obtained from such tumors were consistently free of expression of any SV40 antigens (T, surface and tumor specific transplantation antigens).

The fortitious appearance and detection of marker chromosomes made it virtually certain that tumor was produced by progeny cells of a true subclonal cell, which prior to the injection into the syngeneic mouse was T antigen positive and in fact contained whole (rescuable) SV40 genome. Thus in vivo selection pressures against the SV40 surface and transplantation antigens allowed the selection of a revertant cell. The mechanism of reversion, and whether the reversion is phenotypic or genotypic, is under current study with Dr. Küster (the latter in collaboration with Dr. G. Khoury). By independent experiments with other single cell clonal isolates, both from the T AL/N tumorigenic cells and from other spontaneously and chemically induced tumor cell lines both from the A and from the Balb/c mouse, it appears that the above results are broadly applicable to other genetic systems. Thus complete rejection of SV40 transformed tumorigenic cells can be achieved in other hosts than the AL/N mouse. We are also studying if true revertant (SV40 negative) cells can be selected out by the above technique generally. Thus the in vivo selection method as exemplified above, when applied to tumorigenic cells which became malignant spontaneously or by some other means, and subsequently were transformed by immunogenic tumor viruses, may allow studies on genetic and cellular factors which control the balance between immunogenicity and tumorigenicity, and probably also on the mechanism of excision (or repression) of a virus genome.

Significance to Biomedical Research and the Program of the National Cancer Institute:

Studies of cell surface biochemical changes which relate to acquisition of tumorigenic potential or to immunologic recognition and rejection in vivo are of interest, especially when they may lead to understanding of changes in molecular mechanism which appears to be reducible to heritable changes on control processes on the DNA level in the virally induced transformation of cells.

Proposed Course of the Project:

There will be an attempt to further clarify the relevance of the various changes in cell membrane biochemistry (glycoproteins, proteins, etc.) to spontaneous and to viral induced transformation of cells. We also plan to relate these changes to phenotypic changes in cell immunogenicity and in tumorigenicity in various syngeneic and allogeneic mouse systems. We hope that the information and methodology developed in the mouse system will be of use on human tumor cells.

Publications:

Pancake, S. J. and Mora, P. T.: Limitations and utility of a cytolytic assay for measuring Simian Virus 40-induced cell surface antigens. Cancer Res. 36: 88-94, 1976.

Mora, P. T. (Ed.): Cell Surfaces and Malignancy. Fogarty International Center Proceedings No. 28, Washington, D. C. U. S. Government Printing Office, 1976.

Mora, P. T.: Cell transformation in culture: Pertinence to malignancy: Summary of status and prospects. In Mora, P. T. (Ed.): Cell Surfaces and Malignancy. Fogarty International Center Proceedings No. 28, Washington, D. C. U. S. Government Printing Office, 1976, pp. 247-254.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05543-08 LCBGY

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The role of lysyl residues in intersubunit interactions in L-asparaginase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Shifrin	Chemist	LCBGY DCBD NCI
OTHER:	C. Parrott	Chemist	LCBGY DCBD NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to examine the chemistry of L-asparaginase from several bacterial sources and to determine the role of specific amino acid residues in subunit interactions. The topics of present interest are: 1) The reaction of 2,4,6-trinitrobenzene sulfonic acid with amino acid side chains and subsequent treatment of the trinitrophenylated amino acids with borohydride; 2) Trinitrophenylation of L-asparaginase from Escherichia coli and Erwinia carotovora; 3) The effect of trinitrophenylation of intersubunit interactions; 4) The effect of borohydride treatment on intersubunit interactions in TNP-asparaginase.

Project Description:

Objectives: The purpose of this project is to determine the role of the lysyl residues of L-asparaginase from E. coli and from Erwinia carotorora in maintaining the conformation of the tetrameric protein. These studies were carried out prior to conjugation of the enzyme with a glycopeptide.

Methods Employed: Visible and ultraviolet absorption spectroscopy, fluorescence spectroscopy, sedimentation velocity ultracentrifugation, chemical modification of amino acid residues, polyacrylamide gel electrophoresis, thin layer chromatography, chemical identification of reporter groups and enzymatic assays.

Major Findings:

A. Substitution of 15% of the lysyl residues of E. coli L-asparaginase with 2,4,6-trinitrophenyl moiety causes a 67% loss of catalytic activity of the enzyme. Nearly 88% of the enzymic activity is lost when 22% of the lysyl residues are modified by reaction with 2,4,6-trinitrobenzene sulfonic acid. Studies of rates of dissociation in 4M urea suggest that the chemical modification introduces a bulky hydrophobic residue which distorts the protein conformation which in turn causes a loss in catalytic activity.

Chemical modification by trinitrophenylation produces an electrophoretic variant which is readily distinguished from the unmodified enzyme and thus permits the study of intersubunit interactions through the preparation of hybrid tetramers.

Chemical modification of 20% of the lysyl residues of Erwinia carotovora L-asparaginase by 2,4,6-trinitrophenylation has no effect on the enzymic activity of the protein and does not modify its dissociation behavior in 3.8M guanidinium chloride.

Higher degrees of trinitrophenylation of the Erwinia enzymes causes it to precipitate as had also been observed with poly-L-lysine.

B. The 2,4,6-trinitrophenyl moieties of L-asparaginase and of poly-L-lysine were treated with potassium borohydride at pH 7.5. This treatment resulted in the solubilization of the insoluble precipitates of TNP-asparaginase (Erwinia) and TNP-poly-L-lysine. Solubilization is accompanied by the loss of the 346nm-band and the increased absorbance of the long wavelength band with a maximum at 410nm. Borohydride treatment did not produce any changes in catalyzed hydrolysis of L-asparagine. However, the borohydride-treated enzyme from Escherichia coli dissociated in 4M urea more rapidly than the untreated enzyme. Similarly, D-asparagine does not protect the borohydride-treated trinitrophenylated tetramer against dissociation as effectively as it does the untreated derivative.

Borohydride treatment renders the TNP-asparaginase subunits from reassociating into the tetrameric aggregate.

Significance to Biomedical Research and the Program of the National Cancer Institute:

An understanding of the structural features of proteins which are essential for functional activity will provide a basis for modifying chemotherapeutic enzymes which will enhance their pharmacologic effectiveness. Modification of certain groups may reduce the side effects elicited by L-asparaginase without destroying its catalytic activity.

Proposed Course of the Project:

Conjugation of the glycopeptide isolated from orosomucoid to the ε-amino groups of lysine will be studied in an attempt to determine the role of the sugar moieties on the aggregating properties of the tetrameric enzyme. Studies are being initiated into an examination of the glycoprotein content of cell membranes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05544-08 LCBGY

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cell Surface Changes in Transformed Mouse Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Luborsky	Chemist	LCBGY DCBD NCI
OTHER:	S. J. Pancake	Guest Worker	LCBGY DCBD NCI
	J. M. Coll	Visiting Fellow	LCBGY DCBD NCI
	C. Chang	Visiting Fellow	LCBGY DCBD NCI

COOPERATING UNITS (if any)

J. Anderson, LMB, NIAMDD

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

To study surface changes in transformed cells and the molecular mechanisms responsible for antigenic changes and/or the transformation to malignancy, either spontaneously or as induced by SV40 virus. To develop methods to isolate and purify cell surface membranes, and antigenic components such as SV40 virus specific surface antigens and tumor specific transplantation antigens.

Project Description:

Objectives: As part of a study of surface changes in transformed cells we wished to develop methods to isolate cell surface membranes, to compare those isolated from normal, spontaneously and viral transformed cloned and mass cell lines; and to solubilize and characterize the antigens from SV40 transformed cells.

Methods Employed: Tissue culture cell cultivation, infection with SV40 virus, cell cloning procedures, polyacrylamide gel electrophoresis, column chromatography, ultracentrifugation.

Major Findings:

I. Comparison of surface membranes of normal, spontaneously and viral transformed mouse cells.

Surface membranes from cells in logarithmic growth were compared from a closely related group of mouse fibroblasts. These cell lines included normal, spontaneously and SV40 virus transformed cells, and clones and subclones from them, all of which had approximately equal tissue culture growth rates. We isolated a partially purified membrane fraction from the cell homogenate by a previously described, simple, rapid, reproducible technique adapted in our laboratory for the AL/N derived cells. This procedure involves swelling the cells in a hypotonic medium, breaking them with a Dounce homogenizer, and collecting the membrane fraction on a sucrose solution interface. Cells were labelled chemically by reacting the external, accessible protein amino groups of the cell surface with pyridoxal phosphate, then reducing the resulting Schiff base with NaB^3H_4. We showed this reaction yielded predominantly surface labelling (up to 80% of the label), and that the cells remained viable through the entire procedure (up to 90% viable, by trypan blue dye exclusion). The composition of the surface membrane fraction obtained in this manner was determined by SDS-polyacrylamide disc gel electrophoresis. In spite of the inherent variability of disc gels, careful analysis of many patterns, relative to the protein standards added to each sample, indicated that in the 200,000 molecular weight region of the gel, all SV40 transformed (T antigen positive) cell lines (mass or cloned) exhibited a small but consistent reduction in content of labelled protein from their surface membranes, as compared to those from both normal and spontaneously transformed cell lines (T antigen negative) (with S. J. Pancake).

Analysis of the isolated membrane fraction on slab gels rather than on disc gels improved the reproducibility and sensitivity of the technique; many samples can be run side by side on the same gel at the same time. Staining the proteins with Coomassie blue on the SDS-polyacrylamide slab gels showed only minor, reproducible changes, in one component in the 180,000 molecular weight region. In the T antigen positive mass cell line, SV AL/N, this component was missing or much reduced compared to the normal T antigen negative cell line, N AL/N, while the cloned lines, whether T antigen positive or negative, showed a somewhat reduced intermediate amount of this component.

We labelled cell protein metabolically for 7 hours with a ^{14}C-amino acid mixture and found the T antigen positive cell lines, both SV AL/N and subclone 1 to be much reduced or lacking in this 180,000 molecular weight species, while the T antigen negative cell lines, T clone 3 and subclone 5, clearly possessed lower amounts of this component compared to the original normal N AL/N cell line. These same differences between these cell lines were seen when the slab gels were analyzed by autoradiography.

Thus, we have compared surface membranes from these cell lines by three different techniques: chemical labelling from the outside of the cell; metabolic labelling during cell growth; and staining for protein and autoradiography on the SDS-polyacrylamide slab gels. These techniques all showed the same results. These cells, in logarithmic growth, showed a similar difference in surface membrane composition - a reduction in a 180,000 molecular weight component. Whether this result is due to their growth stage or to some aspect of their transformation by SV40 virus remains to be seen. Confluent cell cultures are currently being analyzed. These results suggest, however, that the 180,000 molecular weight component is probably not related to the possession of T antigen, since some cell lines possessed one but not the other (with J. M. Coll).

II. Solubilization, characterization, and the relationships among antigens from SV40 virus transformed cells.

Surface membrane components were extracted from various SV40 transformed mouse cell lines in logarithmic growth phase, by solubilization with 0.5% Triton x-100 in TBS. The extract was active both in vitro, in inhibition of antibody-mediated cytotoxicity, and in vivo, in immunization of mice against subsequent challenge with syngeneic SV40 transformed tumor cells. The extract was fractionated by ammonium sulfate (AS) precipitation, into three fractions. TSSA and TSTA (as well as T antigen, when extracted - see below) were found in both 0-30% and 30-50% AS precipitates; fractions obtained above 50% AS were inactive. Further fractionation of the 30-50% AS material on Sephadex G-150 yielded two peaks, both containing TSSA and TSTA activities. One, of high molecular weight, eluted with the void volume; the other, near 45,000 apparent molecular weight. Control experiments in which spontaneously transformed AL/N mouse cells or normal AL/N mouse brain cells were used for extraction did not show either activity. Clearly, both TSSA and TSTA are solubilized by detergent extraction.

Additional purification and characterization techniques have been applied to these extracts. Ultracentrifugation of the 30-50% AS fraction through 5-20% sucrose gradients gives predominantly a single peak of TSSA activity sedimenting at about 6.2s, while similar centrifugation of the combined 0-50% AS fractions gives a broader TSSA peak, at 4-6s. In these experiments, the activity peak was always displaced from the protein peak, thus providing the basis for purification of these active fractions. Ion exchange chromatography provided further fractionation and purification of these antigens. On both DEAE-cellulose and on phosphocellulose columns the 0-50% AS fraction was split into two protein fractions, one which did not stick to the columns and

one which did. The TSSA was found only in the material which was absorbed to the columns, as was T antigen when it was present in the extract. TSTA assays are currently underway with this material.

Modification of the extraction to follow procedures previously reported by others has also been done; this retained both TSSA and TSTA activities and added the SV40 T antigen activity to the extract. Thus, three of the major SV40 specific antigens are available for study: T antigen, detected by complement fixation assay; tumor specific surface antigen (TSSA), detected by inhibition of cytotoxicity tests; and tumor specific transplantation antigen (TSTA), detected by tumor rejection tests.

Significance to Biomedical Research and the Program of the National Cancer Institute:

The properties of the cell surface, its chemical composition, structure and antigenic determinants exert important influences upon cell growth characteristics and the ability of certain cells to form tumors. Studies of cell surface biochemical changes which relate to acquisition and expression of tumorigenic potential or to immunologic recognition and rejection in vivo are therefore of particular interest. Since we have solubilized three SV40 specific antigens we can study the correlation of in vitro cytotoxicity and in vivo transplantation-rejection assays as well as the molecular nature of these antigens.

Proposed Course of the Project:

Extracts will be prepared from the isolated surface membrane fraction and assayed for the SV40 antigens. Purified antigen preparations will be used to raise antibody for the preparation of affinity columns to more rapidly and quantitatively prepare a desired purified SV40 antigen for further study. We will attempt to better understand the relevance of the various changes in cell membrane biochemistry and immunochemistry to spontaneous and to viral induced transformation of cells. We will examine the molecular properties and interrelationships of the three SV40 specific antigens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05550-08 LCBGY

PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Etiologic and Immunologic Role of Type-C RNA Viruses in Reticulum Cell Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. S. S. Chang	Medical Officer (Res.)	LCBGY NCI
OTHER:	L. W. Law	Chief, LCBGY	LCBGY NCI
	W. Liu	Visiting Fellow	LCBGY NCI
	M. J. Rogers	Senior Senior Staff Fellow	LCBGY NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Cell Biology

SECTION Viral Oncogenesis Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.50	2.00	1.50

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of the present project is to investigate the interaction of type-C viruses with lymphoid as well as fibroblastic cells, and to study the relationship between various tumor cell surface antigens and viral genome functions.

The topics of current interest are:

1) The role of endogeneous type-C viruses in the etiology of reticulum cell neoplasms (RCN), particulary those of SJL/J strain of mouse which exhibit a high incidence of spontaneous RCN, and the use of this as a model for study of human Hodgkin's disease.

2) Virologic and immunologic characterization of tumors induced during the course of the above studies, especially a T-cell SJL/J lymphoma cell line (established in culture) and B-cell tumors of BALB/c as well as SJL/J origins classified as RCN.

3) Distinction between viral structural antigens and tumor specific transplantation antigens using nonproducer tumor cells originally transformed by type-C viruses.

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Project Description:

I. As reported previously, an N-tropic D1-MuLV isolated from spontaneous RCN-B of SJL/J mouse induced RCN-B when injected into BALB/c mice 1 day after neonatal thymectomy. However, when injected into non-thymectomized newborn SJL/J mice, D1-MuLV induced only a lymphocytic leukemia which was later shown to be of T-cell origin. Therefore, in the present study, newborn thymectomized SJL/J mice were used for RCN-B induction. It was hoped that reduction of the number of T-cell targets and abrogation of immunological capability by neonatal thymectomy might accelerate the appearance of RCN-B in SJL/J mice after injection of D1-MuLV.

The results obtained after an observation period of 10-11 months on the incidence of RCN, which was histopathologically confirmed, indicated that there was no difference between the inoculated and uninoculated control groups: (7/48 or 14.6% of the inoculated and 5/40 or 12.5% of the control showed RCN). The virus titers in the spleen and lymph node extracts of RCN-bearing mice were generally higher (mean titers: 4.1×10^3 XC pfu/ml in the inoculated group and 2.6×10^3 XC pfu/ml in the uninoculated group) than the non-tumorous mice (mean titers: 1.4×10^1 XC pfu/ml in the inoculated group and 3.7×10^1 XC pfu/ml in the control group). These viruses showed N-tropism as did the D1-MuLV. The association of relatively high titers of the virus with RCN does not necessarily indicate an etiologic role for the viral oncogenesis. However, among various possibilities, it may suggest that in addition to type-C viruses other factor(s) may be required for the development of RCN. In other words, the virus may be a necessary but not sufficient condition for the RCN oncogenesis. Further work is necessary to find these factors interacting with the virus in SJL/J mice.

Although the role of xenotropic type-C viruses in mice is not clear, efforts were made to detect such viruses in D1-MuLV stock with negative results. Further work is still in progress to detect xenotropic viruses in tumor lines which arose in the course of these experiments.

II. During the past few years, we have developed a number of transplantable tumor lines in SJL/J and BALB/c mice which were neonatally injected with D1-MuLV. These include:

D1 - Lymphocytic leukemia induced by D1-MuLV in an SJL/J mouse.

D2 - A subline of D1 which was adapted to in vitro culture as an established line. This tumor was shown to be of T-cell origin (Thy 1-positive by membrane immunofluorescence and cytotoxicity test), producing no type-C particles, but gp 69/71 and p30 positive (gs antigen positive). Since no SJL/J tumor is known to be in culture, availability of this line would provide a stable source of target cells for study of SJL/J tumor immunity, and cell mediated cytotoxicity, etc.

D4 - An RCN-A that arose in an SJL/J mouse injected neonatally with MSV(D1-MuLV). The tumor is transplantable for more than 60 generations. This

is EA-rosette forming (Fc receptor positive), Ig-positive on the membrane, and active as an effector cell for antibody-dependent cell-mediated cytotoxicity. Repeated attempts at culture of this line were unsuccessful.

D7 & D10 - Mammary adenocarcinomas of SJL/J mice which were neonatally injected with D1-MuLV after thymectomy. These contain high titers of D1-MuLV. These tumors may serve as controls for RCN tumor studies.

E1 - RCN-B that developed in BALB/c mice injected with D1-MuLV after neonatal thymectomy. It is producing a high titer of B-tropic virus, E1-MuLV. This is also a B-cell tumor as is D4. No culture has been established.

These tumors are being utilized for study of transplantation immunity in SJL/J and BALB/c mice, and for in vitro cytotoxicity tests. The role of type-C viruses in these tests will be pursued.

III. To distinguish between viral structural antigens and tumor specific transplantation antigens (TSTA) expressed on the membranes of a type-C virus-induced tumor, RBL-5, which was originally induced by Rauscher-MuLV, (R-MuLV), and is still producing the virus, has been used in recent years. The advantage of using another tumor, RBL-3, which was originally induced by R-MuLV but found to produce neither infectious type-C viruses nor mature virion particles by electron microscopy is obvious. This tumor was established as a culture (by Dr. David Trisler) and can induce ascitic tumor in C57BL/6 (B6) mice. The presence of R-MuLV genome in RBL-3 is evidenced by the emergence of infectious R-MuLV after treatment of these cells with 200 mcg/ml 5-iododeoxyuridine (IUdR), or repeated passages in vivo. The cells contain gp69/71, 20-26 ng/10^6 cells, and p30 10-31 ng/10^6 cells as detected by radioimmunoassay, and are gs-positive by complement fixation test.

RBL-3 also exhibited FMR group transplantation antigen. B6 mice immunized with RBL-3 were resistant to challenge with FBL-3 leukemia induced originally by Friend-MuLV, and vice versa; (BALB/c x C57BL/6)F_1 mice immunized with RBL-3 were resistant to challenge by another nonproducer tumor (HFL/d-tc) derived from Friend-MuLV-induced tumor in BALB/c mice (Friedman and Lilly). Furthermore, the crude membrane (CM) of RBL-3 and crude soluble (CS) antigen prepared from the former by limited papain digestion induced immunity in B6 mice against FBL-3 challenge. The CM contained both gp69/71 and p30, but the CS contained no p30. Further work is in progress to purify the TSTA from RBL-3.

The cell membrane antigens present on RBL-3 are complex. By membrane immunofluorescence studies with appropriate antisera we could demonstrate the presence of gp69/71, p30, and an antigen or antigens (presumably non-virion tumor antigen) which reacted only with the syngeneic antisera prepared against the CM of RBL-3. Although this antiserum contained anti-gp69/71 and anti-p30, the activity against RBL-3 surface antigen could not be removed by repeated absorption of this serum with unrelated lymphoid tumor cells such as plasmacytoma C1.18(4) cells expressing these virion ants. This syngeneic anti-RBL-3-CM serum cross-reacted with RBL-5 to a certain extent but still retained its specific activity against RBL-3 even after absorption of the serum with RBL-5. Similarly, the syngeneic anti-RBL-5-CM serum cross-reacted with RBL-3

only at low dilutions. These reactivities were not removed by absorption with B6 fetal tissues. The nature of these RBL-3 specific and RBL- specific antigens needs further investigation.

Thus, in the present system, by using syngeneic anti-CM sera and anti-gp69/71, anti-p30 or broad reacting antiviral sera in parallel, and by using complement fixation, cytotoxicity, and membrane immunofluorescence tests, it has been shown that a tumor specific antigen(s) exists which is distinct from gp69/71, p30 or other virion antigens detectable by these antiviral sera. The identity of this tumor specific antigen with TSTA needs to be established in the future.

It was also found that RBL-3 cells possess Fc receptors as evidenced by EA rosette formation, and their ability to act as effector cells for antibody-dependent-cell-mediated cytotoxicity against ^{51}Cr-labeled sheep RBC. Furthermore, kappa chain of Ig was found on the cell surface as detected by membrane immunofluorescence. The tumors formed by the cell transplant in B6 mice produced reticulum fibers, and were classified as RCN-A.

Therefore, RBL-3, a nonproducer tumor originally induced by R-MuLV, is a B-cell tumor, and availability of this in culture would provide a valuable tool for immunologic and virologic studies of RCN.

Objective: 3

Approach: 2, 4, 5

Publications:

Law, L. W., Appella, E., and Chang, K. S. S.: Biologic effects of solubilized H-2 and tumor-specific antigens. Transplantation Proc. 7: 233-241, 1975.

Chang, K. S. S.: Radiation-enhanced murine sarcoma virus genome rescue activity. Chin. J. Microbiol. 8: 131-141, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05552-08 LCBGY

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mammalian Cellular Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. G. Coon	Research Biologist	LCBGY DCBD NCI
OTHER:	Saverio Ambesi-Impiombato	Expert Consultant	LMBGY DCBD NCI
	G. D. Trisler	Postdoctoral Fellow	LCBGY DCBD NCI
	H. Malech	Research Associate	LCBGY DCBD NCI
	A. L. Huang	Graduate Student	G. W. University
	I. B. Dawid	Carnegie Institution of Washington Dept. of Embryology, Baltimore, Md.	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.3	3.0	2.3

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to conceive, conduct and communicate the results of original research in the area of genetic manipulation of mammalian cells using the techniques of cell fusion, cellular hybridization and cybridization to produce unique material for the molecular genetic analysis of cellular events. Of prime importance in the laboratory now is the genetic system in mammalian mitochondria. The mapping of recombinations between mitochondrial DNA molecules is being studied using restriction enzymes and and cRNA-DNA hybridization. The several distinctive subspecies of mouse are proving to be adequately marked material for such studies. Transformation of sensitive cell strains for antibiotic resistance by DNA from resistant cell mutants is being explored as a means to introduce new genes into mammalian cells so that the impact of such genetic surgery can be evaluated.

Project Description:

During the past year profound changes have occurred in the program of this cellular genetics unit. Of the several problems discussed in past annual reports only one principal one is now the prime concern of the laboratory. We are concentrating nearly exclusively on mitochondrial genetics studied as an example of the molecular analysis of mammalian cellular genetics. Two hold over problems are being finished up and their publications are now in preparation. They are:

1) The detection and location of specific neuron antigens that are expressed by neuron x fibroblast hybrid cells. Dr. David Trisler is finishing up that problem and plans to leave the laboratory with that problem in July 1976. Rodent neuron x human fibroblast hybrid cells have been obtained that are losing human chromosomes and these hybrid cells have been injected into rabbits, sera collected, and the antisera adsorbed against the human fibroblast parent and the non-neural tissues of the rodent parent. The resultant antisera are reconcentrated and are reacted with various portions of the freshly dissected embryonic nervous system. The freshly dissected fragments or frozen sections of the rodent nervous system are incubated with adsorbed antisera, washed and then reacted with goat anti-rabbit IgG that has been tagged with fluorescine isothiocyanate, then it is washed again and viewed with fluorescence microscopy and/or a combination of phase and fluorescence microscopy. Localization of antibody reaction in particular neurons, especially in the dorsal spinal cord has been observed. It is anticipated that this will prove a valuable and general new method with which to study specificity in the developing nervous system of mammals.

2) The second problem to be finished up within the next six months concerns Dr. Saverio Ambesi-Impiombato's work with the culture of experimentally induced thyroid cell tumors and their normal cell counterparts from the thyroid gland of rats. This effort has taken a little over one year and it has been rewarded with success. We have indeed been able to grow epithelial cells from tumors and normal rat thyroids that are synthesizing rat thyroglobulin. Continuous culture of differentiated cells from the thyroid gland or from thyroid tumors had not been achieved before this work; only short term cell cultures and organ cultures were available for metabolic studies. Our studies presage a great deal of work on the molecular biology and genetics of regulation in the thyroid gland and its tumors which will become the principal work done by the group in Naples, Italy, to which Dr. Ambesi will be returning in the Fall of 1976.

I have determined, however, in spite of the promising results in these two areas that the support for our work in cellular genetics will never be great enough to maintain these programs and that we must choose a single problem and attempt to build a critical mass in that one small area. The area chosen is the continuation of the mitochondrial genetics program begun in 1970 and described in each of the preceeding annual reports from my unit.

In the past year we have enjoyed the collaboration of Dr. Harry Malech from Dr. Nelson Wivel's Laboratory. Dr. Malech has succeeded in repeating the work described the year before by Dr. Jerry Eisenstadt at Yale, that chloramphenicol resistant mutants of mouse cells could transmit the resistance phenotype via fusion with enucleated cytoplasmic fragments termed, "cytoplasts". Dr. Malech has applied this selection technique to examine the question of the ability of intracysternal cytoplasmic A particles to establish themselves in previously A particle negative cells without nuclear support from the infected cell, i.e., A particle rich cytoplasts which also contained the chloramphenicol resistance cytoplasmic marker were fused to A particle negative cells that were chloramphenicol sensitive. The resultant cybrids were selected in chloramphenicol and examined by electron microscopy for evidence of A particles replicating in the previously negative cells. He has succeeded in that effort which implies that the A particle infested cells contain in their cytoplasm (either in A particles themselves or in an associated structure not yet identified) sufficient information to sustain their replication in a previously negative cell.

During this time we have made many other mutants of our own cell lines that are chloramphenicol and mikamycin resistant. These mutants have yet to be characterized as to whether they will be capable of transmitting the resistance phenotype by cytoplast fusion, but their ability to grow in the presence of a fungizone which increases cell permeability suggests that they are mitochondrial rather than permeability mutants. To date our efforts to produce erythromycin resistant mutants have failed due apparently to failure of erythromycin to penetrate into cells to reach the mitochondria. Other antibiotics are under study for their ability to produce antibiotic sensitivity and the appropriate resistance mutants.

We plan to use these mutants for antibiotic resistance to follow up on our previously published studies of recombination in mitochondrial DNA. In particular, we propose to map the recombination of these resistance markers by making hybrid and cybrid crosses between the closely related subspecies of mice. At the level of the restriction enzyme patterns of Eco R1 and Hin III the standard laboratory inbred strains of mice are indistinguishable from one another. However, we have found that several of the subspecies of M. musculus - notably M.m.molocinous, M.m.castaneous, M.m.domesticus, and M.m.caroli, and closely related species, e.g., M. cervicolor, all have mtDNA restriction sites easily distinguished by both Eco R1 and Hin III. We have previously shown that cRNA-DNA hybridization can be used as a sensitive means to discriminate among the mtDNA sequences of three of these subspecies. It is anticipated that the more complex patterns obtainable by digestion with other restriction enzymes such as Hae III coupled with cRNA-DNA hybridization of these fragments (the Southern technique) will allow for mapping and the definitive demonstration of mitochondrial recombination in mammals. A major part of this project will be undertaken in our laboratory as doctoral thesis research by Ann Huang, a student in the George Washington University genetics program. As indicated above a most promising beginning has been made and we confidently expect results in six months to a year.

In other work with the mitochondrial genetics system we have found that NP-40 extracts of cytoplasmic DNA (presumably considerably enriched for mtDNA) from chloramphenicol resistant cells (Malech's MT OC10) may be added to sensitive cell strains (Malech's BU8, 3T3) using the techniques effective in transforming mammalian cells with adenovirus or SV40 DNA or DNA fragments, and that the chloramphenicol resistance phenotype appears with high frequency ($1/10^3$ - $1/10^4$) among the treated cells. Similar extracts from sensitive cells fail to do this. Thus it appears that cytoplasmic DNA extracts from resistant cells are capable of transforming chloramphenicol sensitive cell strains. The word "transformation" is being taken here in its older and now less familiar sense as it was used in the study of DNA mediated genetic change in Pneumococcus, in the sense of altering the genetic material of the sensitive cell strains so that they become heritably resistant cells. It is tempting to speculate that what has happened in our experiments is that the portion of the mitochondrial (?) genome which can confer chloramphenicol resistance has either repopulated the sensitive cells' mitochondria or has somehow become integrated (perhaps via recombination) into the sensitive cells' mtDNA and thus achieved true transformation of a mammalian gene.

To date, (3/76), two successful experiments have been done in which DNA preparations from resistant cells have produced resistant cells among the progeny of treated sensitive cells. We are now attempting to quantitate and to characterize the DNA which is effective in this process from our extracts. We are starting with the assumption that the effective DNA will be mtDNA. There is, however, no evidence that this is true since our experiments have been performed with only partially purified cytoplasmic DNA and not with purified mtDNA.

If we have succeeded in transforming a mammalian gene, as it now appears we have done, then this will become the first example of a mammalian gene that can be transformed using chemically purified DNA alone. By using the restriction enzyme techniques that have proven effective in E. coli, it should be possible to isolate the active portion of the mitochondrial genome and use it as a vector to transfer other genes into the mitochondrial genome. In effect we shall be able to effect genetic surgery in mammalian cells - at least to produce genetic prostheses for mammalian cells. It is an imperative of our time that we follow up this work vigorously because we need to study the effects of genes artificially introduced into mammalian cells. The mitochondrial system appears to have advantages of multiplicity (2000 genomes per cell) and simplicity (easily isolated from nuclear genes and small size, 10^7 daltons) which the obviously dangerous strategem of using portions of DNA viruses as vectors for nuclear genes cannot offer.

Publications:

Lieber, M. M., Sherr, C. J., Todaro, G. J., Benveniste, R. E., Callahan, R. and Coon, H. G.: Isolation from the Asian mouse Mus caroli of an endogenous Type C virus related to infectious primate Type C viruses. Proc. Nat. Acad. Sci. USA 72: 2315-2319, 1975.

Green, L. A., Shain, W., Chalazonitis, A., Breakfield, X., Minna, J., Coon, H. G. and Nirenberg, M.: Neuronal properties of hybrid neuroblastoma x sympathetic ganglion cells. Proc. Nat. Acad. Sci. 72: 4923-4927, 1975.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05553-08 LCBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure of Mouse Myeloma Proteins with Antibody Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Rudikoff	Microbiologist	LCBGY NCI
OTHER:	M. Potter	Medical Director	LCBGY NCI
	E. Padlan	Visiting Scientist	LMB A
	D. Davies	Chief, LMB	LMB A
	M. Vrana	Research Associate	LCBGY NCI
	D. N. Rao	Visiting Scientist	LCBGY NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Immunochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to perform a detailed study of the primary and three dimensional structure of the antibody combining site. Mouse myeloma proteins with known antigen binding specificity are being analyzed in this regard as well as naturally induced antibodies. This analysis includes a determination of the complete variable region amino acid sequence of these proteins as well as determination of the crystal structure where possible. This study will also provide insight into the genetic mechanisms involved in generation of antibody diversity.

Project Description:

Work for the past year in this laboratory has continued on projects involving an exploration of the antibody combining site. Plasmacytomas of the BALB/c mouse producing homogeneous immunoglobulins which bind chemically defined haptenic determinants have been developed by Dr. Michael Potter. We are currently involved in a determination of the primary and three dimensional structure of groups of proteins that bind the same hapten in order to ascertain the variations in structure within a group of related proteins. Analysis of the primary amino acid sequence of a group of related proteins will also permit an evaluation of the genetic mechanisms compatible with the observed structures. The determination of the three dimensional structure of proteins with different binding specificities will permit an analysis of the degree of change in topography of the combining site with differing specificities.

1. Previous studies in this laboratory have involved the determination of the primary and three dimensional structure of the antibody combining site of a phosphorylcholine binding myeloma protein, M603. Comparative primary structural analysis has also been performed on several other phosphorylcholine binding proteins all of BALB/c origin. Having determined the structure of phosphorylcholine antibodies in BALB/c mice we are currently attempting to extend this study to analyze induced anti-phosphorylcholine antibodies in other mouse strains as well as other species. Preliminary data suggest that certain other mouse strains make the same anti-phosphorylcholine antibodies as those found in the BALB/c mouse. These findings have important implications for proposed theories of antibody evolution and diversity and should continue to shed light on the mechanisms involved.

2. Having determined the three dimensional structure of the M603 protein we are now attempting to perform similar experiments on proteins with other binding specificities. In this regard crystals have been obtained from the Fab fragments of J539 and CBPC4, two galactan binding proteins and EPC109 a levan binding protein. Data collection has been made for the J539 protein and a low resolution x-ray map should be available in the near future. To permit construction of a three-dimensional model of this, protein studies to determine the amino acid sequence are in progress. Approximately 60% of the light chain sequence has been completed including two of the three hypervariable regions which are known to contribute to the antigen binding site. Studies on the heavy chain have defined about 90% of the variable region sequence. These data along with future high resolution x-ray analysis will permit the construction of a molecular model and the comparison with the M603 structure.

Studies are also in progress to determine the amino acid sequence of several other galactan binding proteins. We intend to ascertain if the type of variation seen among phosphorylcholine binding proteins is also true for galactan binders. Again, this should provide a further understanding of the mechanisms involved in the generation of antibody diversity.

3. A final group of proteins selected for comparative structural studies are a series of immunoglobulins which react with levan containing antigens. More than ten of these have been identified and members of this group have been shown to possess shared antigenic determinants (idiotypes) in their variable regions. To date, nearly complete sequences have been obtained from the heavy chains of three of these proteins. Experiments are currently being conducted to provide the methods for isolation of the appropriate peptides from the light chains and their subsequent sequencing. This set of proteins will provide data involving a third binding specificity and thus hopefully permit a final analysis of variation of structures among related antibodies.

The studies described above have led to a continuing attempt at the in vitro synthesis of antibody molecules with M603 as a model. An understanding of the primary and three dimensional variations of antibody molecules by studies such as those described above may eventually permit the in vitro design and synthesis of antibodies with pre-determined specificity.

Relationship to National Cancer Plan:

Objective - 6

Approach - 1

Publications:

Rudikoff, S. and Potter, M.: Size differences among immunoglobulin heavy chains from phosphorylcholine binding proteins. Proc. Natl. Acad. Sci., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 05596-08 LCBGY

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Pathogenesis of plasma cell neoplasia in mice: immunochemical characterization of antigen-binding myeloma proteins; genetic basis of immunoglobulin diversity.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Potter	Medical Director (Res.)	LCBGY	NCI
OTHER:	S. Rudikoff	Microbiologist	LCBGY	NCI
	M. Vrana	Res. Assoc.	LCBGY	NCI
	C.P.J. Glaudemans	Chief	LC	NIAMDD
	D. R. Davies	Chief	LMB	NIAMDD
	E. Padlan	Visiting Fellow	LMB	NIAMDD
	W. P. Rowe	Chief	LVD	NIAID
	R. Risser	Postdoctoral Fellow	LVD	NIAID
	R. Asofsky	Chief	LMI	NIAID
	R. Tigelaar	Medical Officer	LMI	NIAID
	H. Morse	Medical Officer	LMI	NIAID
	B. Mathenson	Guest Worker	LMI	NIAID
	R. Lieberman	Senior Investigator	LI	NIAID
	E. A. Kabat	Consultant	NIH	

COOPERATING UNITS (if any)
NIAMDD, NIAID and University of Maryland

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Immunochemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.00	3.00	4.00

SUMMARY OF WORK (200 words or less - underline keywords)

The role of peritoneal granuloma inducing agents (mineral oil, pristane); endogenous and exogenous (Abelson) murine leukemia viruses; and specific genes in the pathogenesis of plasmacytomas in BALB/c mice is being studied. The antigen-binding activities of myeloma proteins are being determined. Genetic markers (idiotypes, structural properties, and V-region phenotypes) associated with specific antigen-binding myeloma proteins or induced antibodies are used to identify and map immunoglobulin V-region genes.

Project Description:

I. Pathogenesis of Plasma Cell Tumor Development.

A. Genetics of Susceptibility to Plasmacytomagenesis in BALB/c:

Susceptibility to oil-induced plasmacytoma development in BALB/c is multifactorial. The strain dependence however suggests that specific genes can be identified. The differences between BALB/c and the resistant strain C57BL/Ka is being studied.

Analysis of the Bailey RI strains derived from crosses between BALB/c and C57BL/6 revealed two strains that were similar to BALB/c: CXBG and CXBJ. The only genes these mice had in common that were absent in the other five RI strains were the IgC_H allotypes that resembled those in BALB/c. The allotype locus in the mouse is known to be linked to V_H genes and thus the influence of the immunoglobulin structural genes in plasmacytoma susceptibility is suggested. Currently the susceptibility of F_1 hybrids of a^1 a^2 (C57BL/Ka x BALB/c) x C57BL/Ka mice segregating for allotypes a^1 and a^2 will be tested for susceptibility to pristane induced plasmacytomagenesis.

B. Chemical Co-plasmacytomagenesis:

If BALB/c susceptibility to plasmacytoma development is dependent in part on a genetic property of a gene exposing itself in the B-lymphocyte-plasma cell lineage, then this phenotype could be augmented by somatic mutations. Assuming a 2-hit genetic change one coded by a germ-line gene the other augmented by a somatic event, it should be possible to increase plasmacytoma development, by exposing BALB/c mice to mutagenic agents. In early pilot studies using Ethylmethane Sulfonate plasmacytoma development was accelerated. Attempts to extend, amplify, and coordinate this finding with other mutagens are still in progress; currently Ethylnitrosourea is being tested. In two experiments in progress, a more discriminatory protocol is being tested in which ENU treatment is given before injection with 0.5 ml pristane. Thus far, ENU is producing lymphomas (predominantly T-lymphomas) in BALB/c mice, but has not produced an increase in plasmacytomas. In these experiments care must be taken to avoid toxicity, which can in itself modify susceptibility.

C. Abelson Murine Leukemia Virus (MLV-A):

MLV-A virus contains a complete replicating unit, the Moloney Leukemia Virus, and a replication defective component which is responsible for the transforming properties of the virus. MLV-A is capable of infecting adult BALB/c mice and inducing non-thymic lymphoreticular tumors very rapidly i.e. within 21 to 100 days. We are continuing to characterize the tumor types in BALB/c mice and other related strains of mice, the Bailey RI strains. When the virus is injected into the peritoneal cavity of mice that have previously received an injection of pristane, the principle neoplastic type obtained are undifferentiated lymphosarcomas (LS) which are able to synthesize and produce small amounts of immunoglobulin, chiefly IgM that is deposited in the cell

membrane. This group of tumors comprises 80% of all the tumors produced in BALB/c. In addition there are more differentiated tumors of the immunoglobulin producing series including plasmacytomas and plasmacytoid-lymphosarcomas (PL) (probably a cell quite similar to the chronic lymphocytic leukemia cell in man) in its level of differentiation. We are currently attempting to develop a method for rapidly characterizing these lymphocytic tumor types so that lymphocytic tumors can be classified. Preliminary studies indicate that the PL tumors have more immunoglobulin on their surface than the LS tumors. LS and PL tumors are being screened by the rosette method for their ability to bind antigens; SRBC coupled with phosphorylcholine, galactan, bacterial levan, S. tranoroa lps, TNP and dextran are being used as probes. These activities are the most common antigens involved in BALB/c myeloma protein binding.

The genetic susceptibility of adult mice to infection and tumor development is highly strain dependent. BALB/c is uniquely susceptible to MLV-A, C57BL/6 and resistant to most other strains. In collaboration with Drs. Rex Risser and Wallace Rowe we are studying the genetics of susceptibility of the 7 Bailey RI strains (derived from BALB/c and C57BL/6) to MLV-A. Thus far the pattern of LS susceptibility is not the same as for plasmacytoma development. The most MLV-A susceptible strains are CXBD, CXBH (both $H\text{-}2^{d}$) CXBJ and CXBI. Thus far we cannot draw any conclusions about the numbers of genes controlling susceptibility. H-2 appears to play a significant role but in addition there is another major gene. We are attempting to determine how many genes by an analysis of the susceptibility of a backcross population.

We have observed some unusual tumors in several of the Bailey RI strains. Mastocytomas have been found in relatively high frequency in CXBD mice infected with MLV-A and given pristane IP. In one series 20% of the mice developed mastocytomas.

The ability of MLV-A to induce a small number of plasmacytomas in pristane primed mice is being pursued. We have had difficulty in obtaining plasmacytomas in 15 to 20% of the mice as originally reported. This is probably due to our lack of understanding of the proper dose of virus and ability to obtain a virus with the correct ratio of the two elements. Most of our recent virus preparations have been extremely potent and such preparations increase the incidence of lymphosarcomas to nearly 100% and no plasmacytomas are obtained. New preparations of virus are being systematically tested. This problem is considered to be a central one since in theory the conditioned environment effect is not a strain specific, limiting factor governing susceptibility. Thus genetic susceptibility to mineral-oil or pristane induced plasmacytomas in BALB/c must involve an intrinsic property of the plasma cell itself. It has been postulated by some that endogeneous C-type RNA viruses may play a role in plasmacytomagenesis; the infectious MLV-A agent can obviously supply transforming virus. If the type of endogenous C-type RNA virus is critical for BALB/c plasmacytomagenesis and other strains are resistant because they lack the specific virus or some property of the virus, then the infectious MLV-A should be able to bypass the block and induce tumors in plasmacytoma resistant strains. The only useful MLV-A susceptible strains so far

identified, in this respect are CXBH and DBA/2 and (BALB/c x DBA/2)F_1 hybrid mice. Thus far we have not obtained plasmacytomas in these mice with MLV-A but these challenges have not been deemed adequate because of the inability to obtain high yields of plasmacytomas (i.e. 15%) in BALB/c controls.

D. Abnormal Tissue Microenvironment:

A major influence in plasmacytoma development in the mouse is provided by the peritoneal oil granuloma. It was previously shown that the growth of primary plasmacytomas in the peritoneum depended upon the presence of granulomatous tissue. This year we have extensively studied the biological basis of this dependence in collaborative studies with Mr. Michael Cancro (graduate student at the University of Maryland, who is a temporary employee at the NCI). The conditioned environment is established very rapidly after the administration of pristane. Mice injected intraperitoneally with pristane are conditioned within three days. If mice are treated with hydrocortisone simultaneously with the oil, the conditioned environment is weakly established if at all. This appears to be due to the failure of sufficient adherent cell types (monocytes and PMNL) to enter the peritoneum. If, however, the hydrocortisone treatment is begun 3 days after the pristane the conditioned environment is established. These findings indicate that the free pristane which is not removed from the peritoneum is not responsible for the conditioned environment and that the migration of an adherent cell population is an essential step. Further hydrocortisone is not toxic for primary plasmacytoma cells. The single injection of pristane intraperitoneally to mice elevates the adherent cell population in the peritoneum over a sustained period of 50 or more days. A similar increase can also be obtained with the chronic administration of thioglycollate (TGM) medium. However, repeated injections of TGM do not produce a conditioned environment for primary plasmacytoma growth, nor do they establish a granulomatous tissue in the peritoneal connective tissue. This indicates the requirement for a resident granulomatous tissue for the conditioning effect. Since plasmacytomas arise in this tissue it would appear that essential steps in the neoplastic development are completed through adherence on the granulomatous substratum. An *in vitro* correlate of this effect has recently been found - If primary plasmacytoma cells plated in Falcon tissue culture dishes adhere inefficiently, these same cells adhere avidly on a plate containing either peritoneal macrophages or 3T3 cells. Comparatively, established transplantable plasmacytoma cells adhere to Falcon control and macrophage containing plates equally well. This indicates that the plasmacytoma cell *in vitro* requires either the cells or a product of the macrophages or 3T3 cells for adherence.

The demonstration of this adherence phenomenon in experimental myeloma suggest the importance of applying the same type of tests to human myeloma. Here the spontaneous disease develops primarily in the bone marrow cavities. The multiple sites of development suggest that the cells are seeded into the marrow. From the circulation we postulate the marrow is the counterpart for the conditioned peritoneum and that this may sustain the development of the human myeloma cell. Also this finding reopens an important issue which will now be investigated in some depth. Mainly, can the consumption of mineral oil,

in many, result in deposits of oil and local granuloma formation in the marrow which may act as foci for plasmacytoma development? Early work by Boitnott and Margolies indicated that mineral oil deposits were frequently found in human tissues. Further pristane has been found in human tissues. It is very unlikely these are factors in human myeloma but this possibility should be investigated.

II. Immunochemistry of Myeloma Proteins.

We are continuing to produce additions to the collection of myeloma proteins that increase the number of examples with a similar binding activity. There were two new B2,1 fructosan binding proteins TEPC957 bringing the list of transplantable tumors in this group to 7. These proteins are being produced in large quantity for sequencing by Drs. Mark Vrana and Stuart Rudikoff. The ABC48 B2,6 fructosan binding protein has been isolated by affinity chromatography in collaborative studies with Dr. Glaudemans and has also been provided for sequencing.

Alpha 1,6, dextran binding proteins have been isolated by affinity chromatography and the L and H chains separated. L and H chains of QUPC52 and W3129 are being provided for sequencing studies. These two proteins differ remarkably in the size of their binding sites. Sequences of these proteins should provide data for constructing hypothetical 3 dimensional models and confirmation of the predictions by Cisar and Kabat on the differences in the topography of the binding sites.

In collaborative studies with Dr. Davies' group proteins have been provided for crystallization studies. Three new proteins have been crystallized and will possibly be useful in establishing electron density maps: the B2,1 fructosan binder EPC109, and the galactan binders CBPC4 and TEPC601.

The detailed sequence analysis of proteins with similar hapten binding activities has proven to be valuable for studying the genetic basis of antibody diversity. Proteins with a common binding activity for homopolymers e.g. similarly linked galactans or levans or dextrans thus far appear to have very similar V-regions. The small differences among these proteins reflected antigenically in their idiotypes and structurally in a few amino acids provide the most closely related functional immunoglobulins available. By studying the extent of variability among these proteins it may be determined whether the degree of variation can be most plausibly explained by either the somatic mutational process or by multiple germ line genes. This year great progress has been made by Dr. Vrana in obtaining comparative sequences of the V-regions from B2,1 binding myeloma proteins. Last year we (Rose Lieberman) reported that there were many different idiotypes among the B2,1 levan binding proteins and that many of these idiotypic determinants were cross specific shared determinants. The structural basis for this relationship is now being determined. Preliminary sequences indicate that minimal sequence differences exist between the V_H and that greater differences may be found among the V_L. It is too soon to establish any general principles other than to indicate the finding will provide great insight into the mechanism of diversity.

In collaboration with Dr. B. Andersson of the Karolinska Institute a new binding activity has been identified in the MOPC520 protein. We have established here a screening panel of 100 to 150 tumors that have been successfully frozen and retrieved from the liquid N_2 bank at the Litton contract site. Dr. Andersson screened these tumors for their ability to agglutinate RBC and chose strain AL/N RBC fortuitously for this purpose. Of 104 myelomas tested one, the MOPC520, strongly agglulinated AL/N RBC. Other RBC also agglutinated were AL/N but a large panel of RBC including C_3H, C57BL/6, C57BL/10D2, B10.A, 4R, 5R, BALB/c, DBA/2, AKR, NZB etc. were negative.

The results indicate this activity is not directed to any known H-2 antigen, but probably to some highly restricted erythrocyte antigen. The relationship of this myeloma protein to antibodies induced to AL/N RBC will be studied along with numbers of other properties. This activity might be very useful in relating the idiotypes of T cell receptors and conventional immunoglobulins.

III. Genetics of Immunoglobulins.

In collaboration with Rose Lieberman a new genetic marker linked to the IgC_H locus has been identified LEV2,1.1. The strain distribution of this marker is unique thus far, and will provide a valuable new marker that is easy to identify. The idiotype is cross specific and is found on most B2,1 binding myeloma proteins and on antibodies raised to Aerobacter levan. It is probably a natural antibody in most strains of mice. The absence of the marker in the C57BL strain permitted the establishment of linkage to IgC_H by its characteristic distribution in RI strains and congenic strains.

Ms. E. B. Mushinski has now established the new congenic strain BC-8 developed by introgressively backcrossing the BALB/c IgC_H (allotype) locus onto C57BL/Ka. This mouse is potentially very valuable in determining the contribution of C57BL V_L chains in immunoglobulins.

In collaboration with Mr. John Slack (GW) from the University of Maryland we are now actively looking for a marker in the V_K chains between BALB/c and C57BL. The first system to be intensively investigated are markers on B2,6 fructosan antibodies. Electrophoresis of L chains of isolated antibodies in the two strains has revealed an electrophoretic difference in acrylamide gels in urea. This is being confirmed by isoelectrifocusing (IEF). A number of other antibodies will be screened for differences using this method should the IEF differences fail. To confirm the acrylamide gel difference, sensitive methods for characterizing L chains from antibodies of BALB/c and C57BL are being developed. One of the aims is to determine the V_{kappa} L chain genetype of the Bailey RI strains.

Publications:

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Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M. and Davies, D. R.: The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. Proc. Nat. Acad. Sci. USA, 71: 4298-4302, 1974.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 32555-01 LCBGY

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Induction of Tumor Growth and Immune Response to Tumor Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. C. Ting	Medical Officer	LCBGY NCI
OTHER:	L. W. Law	Chief, LCB	LCBGY NCI
	E. Appella	Medical Officer	LCBGY NCI
	M. J. Rogers	Staff Fellow	LCBGY NCI
	M. Glaser	Senior Staff Fellow	LCBGY NCI

COOPERATING UNITS (if any)

Laboratory of Immunodiagnosis, NCI; Laboratory of Cellular Metabolism, NHLI; Department of Surgery, Duke University; Biotech Research Laboratories, Inc.

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

SUMMARY OF WORK (200 words or less - underline keywords)

I. Cell-mediated cytotoxic response to syngeneic tumor: By employing the ^{125}IUdR release assay, primary and secondary cell-mediated cytotoxic response could be generated both by in vivo and in vitro methods, and these immunological reactions were found to give very good correlation to the in vivo immunity. This may allow us to be able to further study the mechanisms for the induction of in vivo immunity.

II. Humoral control of tumor growth and immune response: We have found that humoral factors were produced in the tumor bearing hosts of man and mouse, that could promote the tumor growth and suppress immune response. Further attempts are made to isolate and purify these factors. In addition, antisera and immunoregulatory α-globulin were also found to be able to affect the in vivo or in vitro immunological reactions. These studies should help the understanding of the causes for tumor progression, and to develop means to control tumor growth and immune responses.

III. Characterization of soluble antigens: Soluble antigen extracted from FBL-3 cells, a Friend virus-induced leukemia, and gp70 were found to inhibit the cell-mediated cytotoxic reactions to FBL-3 cells, but not by H_2 antigen or p30 prepared in similar ways.

Project Description:

Objectives: The objectives of this project are to study: 1) In vivo and in vitro induction of primary and secondary cell-mediated cytotoxic responses to Friend virus-induced leukemia (FBL-3); 2) Humoral control of tumor growth and cell-mediated immune responses: Isolation and characterization of tumor growth promoting factors and immunosuppressive factors present in tumor bearing hosts; 3) Characterization of FBL-3 soluble antigen and other soluble antigens; 4) Immune response to fetal antigens.

Methods Employed: Tumor cells transformed by oncogenic viruses, chemicals or unknown agents are kept in the tissue culture or as transplanted tumors in mice. The isotopic antiglobulin technique (IAT) was used to perform serological studies. The ^{125}IUdR release assay (IRA) was used to measure the cell-mediated cytotoxic responses. The in vivo tumor immunity was tested by immunorejection experiments.

Major Findings: I. Immune response to fetal antigens: We have previously shown that fetal antigens are different from tumor associated antigens. In the present study, we further demonstrated that immune response to fetal antigens may be genetically controlled. According to humoral antibody responsiveness to fetal antigens, the hosts could be classified into three categories: good responders, poor responders and non responders. The effect of the fetal antigens on the induction of transplantation immunity was not only dependent upon the host responsiveness, but was also dependent upon the amount of a fetal antigens present in the tumor cells.

II. Specific afferent interference by antiserum of in vivo tumor immunity: We have studied the effect of various antiserum, whose specificity have been well characterized, on the immunogenicity of FBL-3 cells to induce in vivo transplantation immunity. The antisera used in these experiments included: anti Friend leukemia sera, anti SV40 tumor sera, and anti fetal sera. It was found that induction of in vivo tumor immunity could only be specifically blocked by the antiserum with appropriate antigenic specificity. These studies not only showed that antibodies could play some role in in vivo immunity, but also offered a simpler method to define the specificity of tumor associated transplantation antigen.

III. In vivo and in vitro induction of cell-mediated cytotoxic responses: We have modified the IRA for use with suspension target cells to measure the cell-mediated cytotoxic responses to FBL-3 cells, a Friend virus induced leukemia in C57BL/6 mice. By employing this technique, we have been able to detect the primary and secondary cell-mediated cytotoxic response both by in vivo immunization or challenge or by in vitro mixed lymphocyte tumor cells culture technique. The secondary response could be easily distinguished from the primary response in several aspects: it was enhanced (gave higher levels of cytotoxicity), accelerated (peaked at earlier times) and longer lasting. The characteristics of these cytotoxic responses gave a very good correlation to the in vivo tumor immunity: specificity of the reaction, characteristics of the lymphocyte population involved, relationship to the host

immune status (progressors vs. regressors) etc. Thus, it appears that we can use these techniques to study the mechanisms for the induction of in vivo tumor immunity.

IV. Humoral control of tumor growth and immune response: We have found that some humoral factors are produced in tumor bearing hosts that could affect the tumor growth and the host immune response. This is based on the following observations: 1) The tissue fluids obtained from tumor bearing hosts in mouse and man could promote the subcutaneous growth of FBL-3 cells in syngeneic hosts. These tumors usually regressed without the tissue fluids administration; 2) These tissue fluids were able to interfere with the effector phase of cell-mediated cytotoxic reactions (efferent interference); 3) Some of these tissue fluids were able to suppress the induction of cell-mediated cytotoxic responses (afferent interference), thus, it was evident that tumor growth promoting factor(s) (GPF) and immunosuppressive factor(s) (ISF) were present in the tumor bearing hosts. In addition, α-globulin was also found to be able to inhibit the cell-mediated cytotoxic reaction.

V. Characterization of soluble antigens: Various soluble antigens have been used to study their effects on the inhibition of cell-mediated cytotoxic reactions to FBL-3 cells. It was found that soluble antigen obtained from FBL-3 cells could give complete inhibition, whereas the H_2 antigens prepared in similar fashion did not. In addition, the group specific antigen gp70 could give partial inhibition, whereas p30 was not able to do so. These findings indicated that various antigens might be responsible for the induction of cell-mediated immunity, as well as in vivo tumor immunity. Further studies will be carried out to study the specificity of these reactions, and the effect of soluble antigen on the induction of cell-mediated cytotoxic responses.

Significance for Biomedical Research and the Programs of the National Cancer Institute:

Our studies showed that in our experiments, the immunological reactions detected in vitro gave very good correlation to the in vivo tumor immunity. Therefore, if these techniques are properly used, we shall be able to study the basic mechanisms for the induction of in vivo tumor immunity e.g., the roles of humoral factors and immune cells in the induction of immunity, the interactions between different populations of immune cells, and between cells and humoral factors etc.

By examining the tissue fluids obtained from tumor bearing hosts, it was evident that some humoral factors were produced in these hosts that could promote the tumor growth (GPF) and caused immunosuppression (ISF). To study the nature of these factors should help the understanding of the causes of tumor progression. Early detection of these factors in the preclinical stage could be useful for the diagnosis of cancer. Furthermore, finding means to inhibit the production or inactivation of these factors could help to retard the tumor growth.

Proposed Course:

I. To study the mechanisms for the induction of tumor immunity. By employing the techinque which we have already developed, with the adoptive transfer experiments, we shall be able to directly study the mechanism for the induction of in vivo tumor immunity.

II. Characterization of the GPF and ISF: We intend to purifiy these factors with various biochemical or biophysical techniques. These purified materials will be further tested for their effects on tumor growth, and on the induction of cell-mediated cytotoxic responses, and the inhibition of cell-mediated cytotoxic reactions.

III. Characterization of soluble antigen:

Publications:

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SUMMARY STATEMENT
ANNUAL REPORT
DERMATOLOGY BRANCH
DCBD, NCI
July 1, 1975 through June 30, 1976

The Dermatology Branch conducts both clinical and basic research studying the etiology, diagnosis and treatment of malignant diseases involving the skin and host's response to these diseases. The Branch also serves as Dermatology Consultant to all other services of the Clinical Center (approximately 800 patients are seen in consultation each year) and to NIH employees through the Employee Health Clinic. The senior staff of the Branch currently includes the Branch Chief, 6 Senior Investigators, 1 Staff Fellow, 1 Visiting Associate, 2 Expert Consultants, 5 Clinical Associates, and 2 Guest Workers. The Dermatology Branch has made significant contributions to the objectives of the National Cancer Plan. The main research achievements of the Dermatology Branch for the past year are as follows:

T-Cell Leukemia (Sezary Syndrome): A major area of research in the Dermatology Branch is the study of the Sezary syndrome, a lymphocytic leukemia of the T-cell type. The Sezary syndrome is a lymphomatous disease characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood. Findings of the past year include:
(1) A number of patients with cutaneous T-cell leukemia have been treated by leukapheresis. Patients seem significantly improved following treatment, but often relapse requiring further leukapheresis. New chemotherapeutic agents thought to be effective for T-cell lymphomas will be tested by the Medical Oncology Branch in conjunction with leukapheresis for patients with the Sezary syndrome;
(2) It has been found that the Sezary cell has significant B-cell helper function; even being able to "turn on" B cells from patients with genetic diseases which severely inhibit B cell function. In these individuals B-cells can be induced to divide and form immunoglobulins by Sezary cells;
(3) Karyotypes were studied from 11 Sezary patients. Heteroploidy, multiple markers including minute and ring chromosomes, and a lack a modality and clone formation were common chromosomal findings in the syndrome. Abundant spontaneous division of heteroploid cells, a high percentage of heteroploid cells in stimulated cultures, and clone formation were found to be indicators of a poor prognosis for the patient with the Sezary syndrome.

Animal Models for Genetic Human Skin Diseases:
(1) A mutant rat called the vibrissaeless has been found to be the first animal model for human skin blistering diseases. As a result of an autosomal recessive mutant gene product, a suprabasilar separation occurs with what appears to be faulty adhesion of basal cells to prickle cells. The rat also loses its hair;
(2) A mutant rat called masked has been studied and on the basis of an abnormal autosomal recessive gene product, the rat developed crusted lesions on the eyes and nose. Pasteurella organisms have been found in the urine and eyes of these rats.

(3) A mutant mouse called exfoliative has been discovered which suffers a conjunctivitis and exfoliates its skin. Some mutant animals die of what appears to be septic shock. Survivors develop new skin, but suffer intermittant hair loss.
(4) A mutant mouse called motheaten has been studied. The mutant has been found to have hyperimmunoglobulinemia and to die at an early age of autoimmune disease. We have found that survival is not improved by raising the animals in a germfree environment, and also have found peculiar crystalloids in pulmonary cells from these mutants.

Mycotic Infections: A project has been initiated which will attempt to develop a sensitive quantitative assay for superficial fungal growth as a means of measuring cellular immunity and lymphokine formation in patients with mycotic infections.

Xeroderma Pigmentosum: This rare autosomal recessive dermatologic disease is characterized by pigmentary changes, telangiectasia, atrophy and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients (basal and squamous cell carcinomas, malignant melanomas etc.) are histologically identical to those found in otherwise normal persons. A defect (presumably in an endonuclease) in repair of UV damage to DNA has been found in XP cells. The NCI, Dermatology Branch, has the largest series of XP patients on whom complete photobiological, clinical and laboratory evaluation have been performed;
(1) Autoradiographic studies are underway to determine the effects of in vitro ageing of fibroblasts on the DNA excision repair system. Such studies will compare the rates of UV-induced unscheduled DNA synthesis at different passages of certain XP and normal fibroblast strains;
(2) We have studied post-UV colony-forming ability (CFA) in XP fibroblasts from each of the six genetic forms of XP. A striking correlation between the degree of reduction in post-UV CFA and the presence and severity of XP-associated neurological abnormalities has been found. These results constitute the first strong evidence for a relationship between the effective level of DNA repair and the severity of a heredo-degenerative condition of the nervous system. These results support the theory of "intrinsic mutagenesis" which hypothesizes that error-proneness in key DNA repair or replication enzymes is ultimately responsible for a variety of degenerative diseases as well as for normal ageing in various human tissues and for carcinogenesis, especially in the case of those cancers whose incidence increases with age;
(3) We have confirmed recent brief reports that cells from patients with Cockayne's syndrome, a syndrome consisting of cachectic dwarfism, neurological abnormalities and photosensitivity, have decreased post-UV CFA. We have studied cells from six unrelated patients and are establishing additional new strains. The studies have already revealed that there are significant differences among Cockayne strains in the degree of reduction in post-UV CFA. No defects in UV-induced unscheduled DNA synthesis have been found, however in any of these strains. Despite this the probability that these cells do have DNA repair defects is high as one patient in our XP series was found to have the unmistakable clinical findings of Cockayne's syndrome in addition to the signs of XP. Studies in co-operation with Dr. Rufus Day, III, of the NCI are being conducted on the Cockayne cells to determine if these cells have reduced repair of UV-irradiated adenovirus 2. Such a reduction if

found, would clearly indicate the existence of DNA repair defects in this syndrome. Studies in co-operation with Dr. Steven Zimmerman have shown that the levels of DNA ligase activity are normal in Cockayne's cells as well as in XP;
(4) Studies in our lab have confirmed reports of decreased post-X-ray CFA in cells from patients with ataxia telangiectasia, a disease in which patients have an increased rate of cancer, plus degenerative findings in the immune and nervous systems. Such studies are now being performed in parallel with post-UV CFA assays on XP cells, Cockayne cells, and cells from patients with other photosensitive and or degenerative disorders;
(5) We have succeeded in establishing long-term lymphoblast lines from at least one representative of each of the six genetic forms of XP. Work to date with several such lines indicates that they retain the same level of diminished DNA repair as the fresh peripheral lymphocytes from which they arose. Further characterizations of all such lines is underway. These lines should be valuable in attempts to characterize and purify the defective DNA repair enzymes in XP. Efforts are being made to establish such lines from patients with Cockayne's syndrome and ataxia telangiectasia as well;
(6) In collaboration with Dr. Weng-Chow Cheng and Dr. Jacqueline Whang Peng of the NCI, studies of UV-induced chromosome abnormalities and sister-chromatid exchanges are being performed using normal and XP lymphoblast lines. It is already clear that UV-induced gross chromosomal abnormalities and sister-chromatid exchanges are greater in most XP cells than in normals. Comparisons between the different genetic forms of XP are now being made;
(7) A major study of UV mutability of normal and XP fibroblasts is now underway. Two different mutant selection systems are being utilized: Oubain resistance and 6-thiogianine resistance (HGPRT deficiency). These studies will compare mutability among all six forms of XP and will then be extended to include other diseases of interest, such as Cockayne's syndrome;
(8) Attempts are being made to accomplish <u>in vitro</u> malignant transformation of XP and normal fibroblasts of UV light. If successful, the results of such work when correlated with out knowledge of DNA repair efficiency and UV mutability should yield important new information concerning the inter-relationships of DNA repair, mutagenesis and carcinogenesis.

<u>Virology</u>:
(1) A combined biologic, biochemical, and serologic approach is being utilized to study the papilloma viruses associated with human warts. A radioimmune assay for viral antigen has been developed and is being refined. Purification of viral DNA will be accomplished for nucleic acid hybridization studies. These procedures will be used to monitor efforts to propagate virus in tissue culture as well as to evaluate the relatedness of virus in various types of warts. The relationship to other papova viruses will be explored. Skin from a variety of disorders of epidermal proliferation, including cancers associated with <u>epidermodysplasia verruciformis</u>, will be evaluated for the presence and expression of papilloma virus genetic material. Other malignant tissue will be studied virologically;
(2) A sensitive, quantitative assay is being developed to study the biologic effects of the DNA viral intermediate and provirus of mammalian C-type RNA viruses. This procedure will be used to assess the ability of DNA to be infectious and/or to induce cellular transformation. Optimal conditions for extraction and titration of viral-specific DNA are being defined. The effect

of chemical and physical treatment on virus expression will be evaluated. Differences between unexpressed and expressed endogenous and exogenous viral genomes are under study. Preliminary results indicate that DNA from AKR mouse cells (which contain endogenous viral genomes) can be rendered infectious when cells are treated with 5-iododeoxyuridine prior to extraction of DNA, while DNA from untreated cells is not infectious. The physical changes in DNA which underly these biologic differences are being explored.

Immunopathologic Mechanisms Involved in Blistering Skin Diseases: Pemphigus, herpes gestationis, bullous pemphigoid and dermatitis herpetiformis are blistering skin diseases all of which are strongly associated with so-called autoimmune phenomena. Our studies have been mainly concerned with the identification and characterization of serum and in vivo bound antibodies in the latter 3 diseases, centering around the mechanisms of tissue destruction, ultrastructure, and ultrastructural localization of these antibodies. Gastrointestinal abnormalities, which are very similar to those seen in gluten sensitive enteropathy occur in dermatitis herpetiformis and are also under active study. Immunogenetic considerations with regard to HLA associations and the identification of specific B-lymphocyte antigens have provided us with the tools required to study the triggering mechanisms involved in the early stages of an abnormal immune response:

(1) Almost all of 40 patients with dermatitis herpetiformis have in vivo bound IgA and various complement components;

(2) The exact location of these antibodies has been sought and found to localize ultrastructurally in 1) clumps in the dermis, 2) associated with anchoring fibrils in the dermis or 3) along the lamina lucida of the basement membrane;

(3) The early ultrastructural destruction of the skin in these patients has been demonstrated to correlate well with the antibody localization;

(4) Almost all patients with dermatitis herpetiformis have morphologic and enzymatic evidence of gastointestinal abnormalities very similar to those seen in patients with gluten-sensitive enteropathy;

(5) In some patients it is possible to not only reverse the gastrointestinal abnormalities but to reduce or completely eliminate their requirement for sulfones by strict adherence to a gluten-free diet;

(6) 85% of 39 patients with immunologically proven dermatitis herpetiformis have HLA-B8 as compared to 20% of controls;

(7) All patients with dermatitis herpetiformis have a specific antigen on their B-lymphocytes. This antigen is not present in the normal population:

(8) A 50% potassium iodide occlusive patch test in most cases produced a lesion histologically identical to de novo lesions in dermatitis herpetiformis;

(9) Sulfones dramatically benefit patients with erythema elevatum diutinum;

(10) By studying five patients with herpes gestationis with immunologic methods we have been able to identify the serum factor responsible for depositing complement at the skin basement membrane zone, the site of earliest destruction;

(11) Ultrastructural localization of complement correlates well with the earliest destruction seen in the electron microscope in herpes gestationis and further strengthens the concept that immunologic mechanisms play an important, if not essential, role in the pathophysiology of this disease.

Studies on Mechanisms of Cellular Immunity in Man: A combination of *in vitro* and *in vivo* controlled methods for the study of cell-mediated immunity in man, have allowed us to initiate basic immunopharmacology and immunogenetics approaches for the study of a variety of diseases and has already resulted in several practical diagnostic procedures. Furthermore, initial studies offer promise of a predictive index for agents affecting the cellular immune apparatus, which is known to be important in host defense against a variety of neoplastic processes:

(1) It was found that human epidermal cells induce blastogenesis in allogeneic leukocytes, whereas, human epidermal cells did not induce blastogenesis in autologous leukocytes nor in the leukocytes of a mixed leukocyte HL-A identical sibling pair. These results indicate that epidermal induced leukocyte blastogenesis is a measure of histocompatibility in humans comparable to a one-way mixed leukocyte reaction;

(2) We have achieved isolation and partial characterization of a factor released by glass adherent phagocytic cells which is an essential requirement for the blastogenic response of lymphocytes to specific antigens, leukocytes, and phytohemagglutinin;

(3) Lymphokine activity has been found in allergic contact dermatitis, there is evidence that such lymphokines may be the mediators of cell mediated immunity;

(4) The DNCB system has offered the versatility that made it possible to show the lymphokines responsible for lymphocyte transformation in target leukocyte populations from nonsensitized subjects were as active on syngeneic (identical twins) leukocytes as on allogeneic leukocytes, indicating the observed activity was not due to histocompatibility factors;

(5) Recent studies indicate that an entire series of DNP containing amino acids induce varying degrees of lymphocyte transformation specifically in subjects topically sensitized with DNCB. The ability to stimulate with soluble DNP containing substances as well as with our previously developed particulate antigen offers great flexibility in our combined *in vitro* - *in vivo* system for investigating the cellular immune response in humans. Furthermore, these studies might prove to be of considerable importance in understanding the mechanism of triggering a specific cell mediated immune reaction;

(6) We have demonstrated the ability of sperm cells to induce allospecific lymphocyte transformation. We have previously demonstrated the ability of macrophages, certain lymphocytes, and human epidermal cells to induce allospecific lymphocyte transformation. We have developed methods for freeze-storing human spermatozoa and preserving their ability to stimulate allogeneic lymphocytes. This methodology, which uncovers the important HL-A-D (LD) locus responsible for graft rejection allows a practical and superior approach to study the genetic locus containing immune response genes associated with a variety of human diseases.

Chemistry, Structure & Biosynthesis of Mammalian Epidermal Keratin Filaments:

(1) The polypeptide chains which comprise the subunits of the keratin filaments of normal bovine epidermis have been isolated and characterized. The polypeptides polymerize *in vitro* into filaments which have the same physical and chemical structure as the *in situ* keratin filaments. The

polymerization in vitro follows an ordered biphasic cooperative mechanism and analysis of the details of this process will provide information on the ultrastructure of the epidermal keratin filament;
(2) The polypeptide subunits obtained from abnormal human epidermis are different from those of normal epidermis, implying the existence of abnormalities in the chemistry and structure of the proteins. Further work is in progress to identify such differences;
(3) Normal mouse epidermal cells grown in cell culture to study the biosynthesis of the keratin proteins do not synthesize keratin. Rather, the cells synthesize large amounts of a muscle actin-like protein. Interestingly, a variety of human and mouse epidermal tumors also contain large amounts of actin-like protein. The possibility of using the actin as a biochemical marker in vivo and in cell culture for studies on malignant epidermal tumor is being investigated.

Applications of Scanning Electron Microscopy to Soft Biological Tissues Including Leukocytes:
(1) Scanning microscope examination of normal human peripheral leukocytes reveals a remarkable similarity among them with regard to their surface morphology. Nearly all lymphocytes, monocytes, and granulocytes appear spherical and covered with uniform short microvilli. Occasional discrete ruffles occur on each cell type. When fixed in suspension in conventional buffy coat preparations or in samples from Ficoll-Hypaque gradient centrifugation, the neutrophils are changed very little, (perhaps with fewer microvilli), but very few lymphocytes retain discrete ruffles, and most monocytes become covered with ridges or ruffles. If lymphocytes are collected on silver filters prior to fixation, as recommended by others, their topography is drastically altered; when collected from Hank's balanced salt solution the majority of normal peripheral lymphocytes become smooth-surfaced, but if 10% fetal calf serum is present, the microvilli on most lymphocytes become exaggerated. These observations account for much of the variation among published accounts of lymphocyte surface form. Thymocytes are generally smooth-surfaced when viewed in situ. The vast majority of circulating lymphocytes are thought to originate (or exist for a significant period) in the thymus. Thus, they must develop microvilli, since virtually all circulating lymphocytes appear villous;
(2) Within one hour after settling on glass coverslips adherent mouse epithelial cells begin to aggregate, and by 12 hours few cells occur individually by 12 hours there was striking organization among the cells with flattened basal cells forming discrete islands against the glass and the other cells clustered on top of them. Between one and seven days in culture increasing numbers of the superficial cells became spherical, smooth-surfaced, and developed pits and performations indicative of degeneration; TEM of vertical sections through these cell clumps confirmed this supposition;
(3) Among six spontaneously transformed lines of mouse embryo fibroblasts of common single cell origin studied by SEM the cells of one line appeared remarkably heterogeneous. These cells produced a tumor which was explanted and derivative cells were maintained in culture for nine passages. By SEM the cells of the tumor-derived line were also heterogeneous and the population was indistinguishable from the parent line. Thus, any or all of the many forms in this line may be tumorigenic, and the morphological heterogeneity

may be considered a phenotypic trait of this line which persists through growth both in vitro and in vivo;
(4) Isotopically labeled cells from suspension which adhere to monolayers of the same cell type can be unequivocally distinguished from morphologically identical cells in the monolayer by autoradiography. This may be the first successful combination of this technique with SEM.

Biochemical Characterization of Mammalian Melanosomes and Enzymatic Control Mechanisms in Melanogenesis:
(1) The melanosome in normal mouse tissue is composed of multiple proteins, many of which are loosely bound and easily extracted. These probably constitute the proteins of the limiting membrane of the organelle. Another protein is of lower molecular weight and is tightly bound to the granule, and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. Melanosomal proteins from melanoma tissues vary in structure from those of normal tissue. Several of the proteins seem to be missing completely from these granules, which are also structurally distinguishable from normal granules by electron microscopy. Perhaps more importantly, many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules. This has been found to be the case in human melanoma as well.
(2) Since only one enzyme (tyrosinase) is essential for melanin biosynthesis, it is a unique system for the study of enzymatic control mechanisms in normal and malignant tissues. We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, but not peroxidase, as has been suggested by some workers. It has always been a subject of dispute how tyrosinase, which can be easily demonstrated to be present in an active configuration in the endoplasmic reticulum and Golgi apparatus, is inactivated in vivo in mammalian systems and subsequently activated once in situ in the melanosome. In amphibians, the mechanism seems to be proteolytic activation; in mammalian and avian systems, however, proteolysis has no function in this regard. It has been found in this study that tyrosinase is under allosteric control and that phospholipids may play a part in the expression of the enzyme's activity. It has also been found that this allosteric regulation in murine melanomas is altered; this perhaps explains the atypical melanosome formation in these tissues.

Surgical Treatment of Darier's Disease (Keratosis Follicularis):
Patients with severe, recalcitrant, confluent Darier's disease are treated with either dermabrasion or dermatome stripping. Rate of wound healing is measured by histologic examination of sequential punch biopsies taken after incisional wounds, wounds made by 3 mm dermal punches, or sites of dermatome stripped skin. Results indicate that surgical treatment can produce low lasting (over 4 years to date) disease free remissions. Furthermore, wound healing, especially epidermal migration, is more rapid in the diseased skin than in uninvolved skin.

Therapy of Skin Cancer and Disorders of Keratinization:
Successful treatment of Basal Cell Carcinomas has resulted from the use of topical Vitamin A Acid, either alone or in combination with liquid nitrogen or topical 5FU. Successful treatment has been achieved in basal cell

carcinoma with topical BCNU, psoriasis with topical CCNU, and ichthyosis with topical alpha-hydroxy acids, and the unsuccessful treatment of psoriasis with topical Vitamin A Acid.

Morphologic and Biochemical Effects of Vitamin A and Analogs on Chick and Human Skin:
Chick metatarsal skin, when cultured *in vitro*, is stimulated to undergo mucous metaplasia by vitamin A acid. Ultrastructural analysis alone cannot determine whether a post-translational effect, a genomic effect, or an effect of vitamin A acid on the mesenchyme is the primary mechanism involved in the inhibition of keratinization and induction of epidermal mucin biosynthesis by vitamin A acid.

A screening System for Topical Chemotherapy of Mycosis Fungoides:
Contracts are currently being awarded to establish a program to topically screen chemotherapeutic agents for possible therapeutic use in mycosis fungoides, and subsequently in benign hyperplastic diseases, such as psoriasis, and in cutaneous carcinoma. After chemotherapeutic agents have been supplied by the DCT, NCI, and after these agents have been screened for percutaneous absorption and skin irritation in experimental models, then clinical testing will be done initially in mycosis fungoides.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3630-06 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Morphologic and Biochemical Effects of Vitamin A and Analogs on Chick and Human Skin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Peck	Senior Investigator	Derm NCI
OTHER:	B. K. Wetzel	Senior Investigator	Derm NCI
	P. M. Elias	Asst. Professor of Dept. of Dermatology Univ. of California Medical Center	
	P. M. Steinert	Visiting Fellow	Derm NCI
	F. U. Lichti	Senior Investigator	Exp. Path.
	S. Yuspa	Senior Investigator	Exp. Path

COOPERATING UNITS (if any)

Experimental Pathology Branch

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.68	0.68	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project proposed to morphologically and biochemically define the mechanism of action of vitamin A acid in altering epidermal differentiation in embryonic skin, normal skin, and in benign and malignant lesions of skin.

Chick metatarsal skin, when cultured in vitro, is stimulated to undergo mucous metaplasia by vitamin A acid. Ultrastructural analysis alone cannot determine whether a post-translational effect, a genomic effect, or an effect of vitamin A acid on the mesenchyme is the primary mechanism involved in the inhibition of keratinization and induction of epidermal mucin biosynthesis by vitamin A acid.

Previous years number 3625-04 reported under number 3630-06 for year 1975 to 1976.

Project Description:

Objectives:

1) To investigate the mechanisms governing epidermal cell differentiation.

2) To morphologically investigate the mechanism by which vitamin A alters epidermal cell differentiation with resultant inhibition of keratinization.

3) To determine if epidermal cells are capable of both mucin and keratin production, or whether the two processes are mutually exclusive.

4) To study the early changes of vitamin A induced mucous metaplasia at the ultrastructural level.

5) To investigate cutaneous macromolecular biosynthesis, and to define the effect of vitamin A in inhibiting keratinization, in embryonic skin, normal adult skin, and in benign and malignant lesions.

6) To define certain dermatologic disorders at the biochemical level.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

1) Metatarsal shank skin (devoid of feathers) is obtained from 13-14 day chick embryo.

2) 0.2-0.4 mm thick, Castroviejo keratotome slices of normal and diseased human skin. Skin from patients with Darier's Disease (NCI-3643) has been used to date.

3) 3-4 mm punch biopsies of normal and diseased human skin (NCI-3643).

Methods Employed:

1) Check metatarsal skin is cultured in vitro and stimulated to undergo mucous metaplasia by vitamin A acid. Specimens are evaluated for mucin production by the use of special strains, such as PAS (with and without diastase), Alcian Blue, Toluidine Blue, and Colloidal iron. Specimens are also examined under the transmission and scanning electron microscopes. In addition, the scanning electron microscope is being used to study in ovo skin scale and feather development, and their inhibition to vitamin A acid in vitro. The effect of retinyl

acetate on the morphology of mouse epidermal cells in culture is also being studied.

2) Chick embryo skin or skin from patients with Darier's Disease was incubated both with and without Vitamin A in a water bath shaker in the presence of labelled precursors. After incubation, specimens were prepared for biochemical extraction and analysis.

Major Findings:

1) Using 20 i.u (6.7 ugm) vitamin A acid per milliliter of culture media, it was found that maximum mucous metaplasia occurred with 3 days of in vitro incubation. As little as 1 I.U. produced metaplasia in 6 days. Metaplasia was inhibited or reduced greatly at room temperature, or in the absence of serum.

2) Microscopically, the control specimen keratinized normally with the formation of thick bundles of tonofilaments, keratohyalin granules and a multi-layered stratum corneum. Vitamin A acid treated specimens did not undergo keratinization. There was no tendency of the columnar basal cells to flatten as they approached the surface. Neither stratum corneum nor keratohyalin granules were formed. Tonofilaments were present in small, find bundles. In addition, there was increased folding of the nuclear membrane, and increased amounts of rough endoplasmic reticulum and polyribosomes. Mucin was present in the cytoplasm as membrane-bound electron dense material. Other transmission E.M. findings included desmosomal cleavage, formation of tight junctions, and basement membrane gaps with elongated processes of epidermal cells extending into the dermis between the gaps. Direct contact between fibroblasts and collagen fibers, with the epidermal cell processes is seen. The germinative cells of the epidermis are small, darkly staining, and increased in number. The more superficial spinous cells are large and lightly staining suggesting a distinct and direct effect on germinative cells.

3) In addition to mucin formation there is a glandular metaplasia in the epidermis with formation of acini. This morphologic change has not been demonstrated previously.

4) Vitamin A induced mucus glandular metaplasia was inhibited by the addition of puromycin or cycloheximide to the culture medium; actinomycin D, hydroxyurea, and colchicine failed to inhibit this process, Thus suggesting that protein synthesis may be necessary for metaplasia, while mitosis, and DNA and RNA synthesis may not.

5) Scanning electron microscopy revealed that metaplastic changes were visible as early as one day after vitamin A exposure. Individual epidermal cells lost attachments to their neighbors, became spherical, and developed surface villi. Selective basal cell layer effect was

observed in 17-day chick embryos treated with vitamin A. Although the superficial stratified squamous layer of the epidermis was unresponsive to vitamin A, it was thrown into large folds by the hyperplastic masses of underlying metaplastic cells.

6) Chick skin scale formation is inhibited by vitamin A acid.

7) We have studied the effects of vitamin A on embryonic chick skin at the biochemical level. DNA synthesis as measured by ^{3}H-Thymidine incorporation declines progressively and becomes negligible by the 6th day after explanation. In contrast to other systems, vitamin A does not stimulate DNA synthesis. On the other hand, overall rate of protein and RNA synthesis is not affected by placing skin in culture. Electrophoretic protein analysis indicates that synthesis of several major polypeptides is turned off by vitamin A administration, but to date, we have been unable to find the stimulation of any protein that might represent the prosthetic group of mucin (a glycoprotein). On the other hand, when H^{3}-fucose (one of the sugars in epithelial mucins) is used as a precursor, it can be seen, again with the aid of gel electrophoresis, that several glycoproteins are decreased in synthesis with vitamin A administration, while one low molecular weight glycoprotein (which is absent in normal tissue) is synthesized very actively ^{35}S-sulfate incorporation into macromolecules of the epidermis, presumably sulfated mucopolysaccharides, is also stimulated by vitamins. While further work is needed, these results would seem to indicate that vitamin A causes mucus metaplasia primarily by inhibiting keratinization, and at the same time allowing mucin formation to occur, presumably by the glycosylation of pre-existing protein in the chick epidermal cell.

8) Preliminary electrophoretic analyses have indicated that several major protein components found in normal skin are lacking in involved skin of Darier's. This data, when considered with the overall hypometabolism found in Darier's patients, might indicate that certain metabolic processes are inhibited in these patients. In response to vitamin A treatment, although the overall rate of protein synthesis was only slightly increased in Darier's skin, whatever metabolic block was functioning in the suppression of the several missing proteins, was effectively removed, and substantial synthesis of these proteins began in these tissues. It has been known for some time that occasional patients with Darier's Disease respond to topical and systemic therapy with vitamin A. This study may provide the first clue as to the mechanism of action of vitamin A in the treatment of Darier's Disease. The identity of these critical proteins remains to be established. We are currently studying the synthesis of the various RNA's in these tissues, and the response of their synthesis to vitamin A treatment, in an effort to determine at what level these inhibitory factors and the depression mechanisms are located. These studies, it is hoped, will indicate the metabolic abnormality in Darier's Disease, and hopefully, an effective way to correct it.

Our results indicate that vitamin A acts at the level of cellular control mechanisms, and, at least in the case of embryonic chick skin, normal and Darier's human skin, causes basic redifferentation of the cell at the molecular level.

Significance to Cancer Research:

1) Since carcinogenesis is an instance of altered differentiation, studies of vitamin A-induced redifferentiation, both in human and chick skin, may serve as an excellent model for investigations of cellular control mechanisms which relate to carcinogenesis. The fact that carcinogen metabolism and carcinogenesis are influenced very markedly by vitamin A deficiency directly links research on vitamin A epithelial effects to cancer research.

2) Furthermore vitamin A is of value in the treatment of malignancy. The mechanisms of action of vitamin A in inhibiting keratinization may be related to its antineoplastic activity.

Proposed Course of Project:

1) In chick skin, continued biochemical analysis will be done in order to more accurately characterize the epidermal mucin formed, and the mechanism by which vitamin A inhibits normal epidermal protein synthesis and stimulates mucin biosynthesis.

2) In Darier's Disease, more patients will be studied to confirm our preliminary observations, and to characterize the major epidermal proteins that are lacking in diseased skin. Skin from normal volunteers may be needed for comparative analysis.

3) Continued ultrastructural examination of the above material.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3659-02 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Therapy of Skin Cancer and Disorders of Keratinization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Peck	Senior Investigator	Derm NCI
OTHER:	F. W. Yoder	Clinical Associate	Derm NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Successful treatment of BCCA has resulted from the use of topical Vitamin A Acid, either alone or in combination with liquid nitrogen or topical 5FU.

Other studies include the successful treatment of basal cell carcinoma with topical BCNU, psoriasis with topical CCNU, and ichthyosis with topical alpha-hydroxy acids, and the unsuccessful treatment of psoriasis with topical Vitamin A Acid. The treatment of a variety of disorders of keratinization with oral 13-cis retinoic acid will begin shortly.

Previous years numbers 3631-05, 3633-04 and 3655-04 reported under number 3659-02 for 1975 to 1976.

PHS-6040

Project Description:

Objectives:

To evaluate safety and effectiveness of a variety of new oral and topical agents in the treatment of skin cancer and disorders of keratinization.

Agents:

1) Alpha-hydroxy acids: 5% lactic acid, pyruvic acid, methyl pyruvate, ethyl pyruvate, tartaric acid or glycolic acid in water washable ointment base.

2) 0.5% to 3.0% Vitamin Acid in water washable ointment base (unibase).

3) 0.4% and 1.0% CCNU in 30% liquid petrolatum and 70% white petrolatum.

4) 0.1% to 0.4% Bischloroethyl-nitrosourea; and 0.1%, 0.4% and 1.0% chloroethyl-nitrosourea, in 30% liquid petrolatum, 70% white petrolatum.

5) 13-cis retinoic acid, 10 mg and 20 mg tablets.

Material:

1) Ichthyosis
   a) lamellar ichthyosis
   b) X-linked ichthyosis
   c) ichthyosis vulgaris
2) Darier's disease
3) Psoriasis
4) Keratoderma palmaris et plantaris
5) Pityriasis rubra pilaris
6) Nevus comedonicus
7) Basal cell carcinoma
8) Nevoid basal cell carcinoma syndrome
9) Xeroderma pigmentosum with cutaneous carcinoma

Methods Employed:

1) Disorders of Keratinization
   a) Topical medication will be applied twice daily for up to 6 weeks or until clear. Occlusive dressings may be used at bedtime.
   b) Serial photographs will be obtained.
   c) Oral 13-cis retinoic acid will be given for 4 weeks at 1mg/kg/day. If no response the dose will be increased for another 4 weeks.

2) Skin Cancer
   a) Patients must have pathologic confirmation of the clinical diagnosis.

b) They must have normal hematologic, hepatic, and renal function.
c) Lesions on eyelids and canthi are not included.
d) Patients are cautioned to avoid sun.
e) Ointments are applied twice daily.
f) Laboratory tests are done before, during, and 6 weeks after therapy.
g) Serial photographs are taken.

Major Findings:

1) Treatment of skin cancer with topical BCNU and CCNU

A total of 12 patients were included in this study. Nine received only BCNU ointment. One received only CCNU ointment; the remaining two received CCNU ointment initially and were given BCNU later as a second course of therapy.

Results of treatment are as follows:

BCNU 0.1% 26 lesions 10 cures 15 partial responses one no response
CCNU 1.0% 7 lesions 1 cure 5 partial responses one no response
Partial response is defined as visible flattening of the tumor mass. The ten BCNU cures were in four patients. To date, neither recurrences nor signs of chronic toxicity have been observed. Severe contact dermatitis has occurred in three patients with 0.1% BCNU ointment. The dermatitis appeared from day 2 to day 33. The main difficulty in conducting this study was the lack of an assay system for the stability of the ointment.

2) Treatment of skin cancer with topical 1% Vitamin A Acid

In one patient, a 4 X 2 recurrent basal cell epithelioma resolved following treatment with topically applied 1% Vitamin A Acid. The lesion had been pretreated with superficial liquid nitrogen cryotherapy 8 weeks previously. No recurrence has developed during the 4 year observation period. In a second patient, 3% Vitamin A Acid was used to successfully treat a nodular basal cell carcinoma on the dorsum of the right hand. In a third patient, a solitary large keratoacanthoma resolved clinically with the combination treatment of topical 1% vitamin A acid and 5% 5-fluorouracil. The lesion recurred, however, within a few months. A patient with the Basal Cell Nevus Syndrome is being treated with the combination of topical Vitamin A Acid and topical 5-FU. Most tumors have responded well, a few have disappeared completely. A patient with a solitary large superficial BCE was treated for three weeks with the combination of topical Vitamin A Acid and topical 5-FU. This lesion underwent complete clinical and histological involution. However, the lesion recurred in one area one year later.

3) Treatment of psoriasis with topical CCNU

Seven of 9 patients treated for longer than 2 weeks cleared com-

pletely. The 2 patients who did not clear had severe psoriasis of the palms and soles. Four additional patients were treated for less than two weeks. Severe irritation of the skin surrounding the psoriatic plaques occurred. This was especially marked in the intertrigenous areas. Subsequent to the cutaneous irritation, persistent pigmentation, telangiectasia, and cutaneous pain developed as well as temporary bone marrow suppression. Two patients have remained free of disease in CCNU treated areas sinct October, 1970, to the present. No signs of delayed or chronic toxicity have appeared.

4) Treatment of psoriasis with topical Vitamin A Acid

No preferential beneficial response was seen in the treatment of 14 psoriatic patients using vitamin A acid cream without plastic occlusion either (1) at a concentration of 0.05% for 12 weeks in a double blind manner against unibase control, or (2) at concentrations varying from 0.1 to 0.3% for 6 weeks at any given dosage level in comparison with a corticosteroid cream. Three of nine patients treated with 0.25% vitamin A acid cream under plastic occlusion in a double blind manner showed definite evidence of clearing limited to vitamin A acid treated areas. This response, which was evident 2 to 4 weeks after onset of therapy, was short-lived in two of these patients, who also failed to respond to subsequent courses of therapy.

5) Treatment of disorders of keratinization with topical alpha-hydroxy acids

a) 80F Psoriasis-scalp - no effect - 3 weeks - methyl pyruvate.
b) 45F Darier's Disease-feet - moderate reduction of hyperkeratosis glycolic acid.
c) 45F Darier's Disease-thight - no effect in minimally hyperkeratotic lesions, glycolic acid.
d) 45M Darier's Disease-thight and chest - moderate reduction of hyperatosis with methyl and ethyl pyruvate.
e) 18M Darier's Disease-chest - no effect on niminally hyperkeratotic lesions with lactic acid.
f) 4M X-linked ichthyosis - complete clearing in 3-4 days. Total body-with glycolic acid - No recurrence in 3-4 weeks.
g) 4F lamellar ichthyosis-neck and shoulders - no effect with ethyl pyruvate without occlusion for 3 weeks.
h) 25F lamellar ichthyosis-total body - moderate reduction of hyperkeratosis using glycolic acid without occlusion. Complete clearing of hyperkeratosis using lactic acid with occlusion - beginning after 1 day of therapy - recurrence seen 4 days after discontinuation.
i) 25M Keratoderma palmaris et plantaris, pyruvic acid and occlusive dressing. No effect.

Significance to Cancer Research:

1) Skin Cancer

a) These are exploratory studies of new drugs in skin cancer prompted by a desire to find a superior topical agent to 5-Fluorouracil in propylene glycol which is in widespread use.

b) We would also hope to contribute to the knowledge of the clinical pharmacology of these agents. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies. It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.

Proposed Course of Project:

1) Treatment of disorders of Keratinization with 13-cis retinoic acid will soon begin.

2) Continued observation of certain patients from other studies.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3643-06 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Surgical Treatment of Darier's Disease (Keratosis Follicularis)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Peck	Senior Investigator	Derm NCI
OTHER:	L. Dellon	Surgery Branch	NCI
	P. Chretien	Surgery Branch	NCI
	I. Cohen	Surgery Branch	NCI
	W. G. Klingler	Clinical Associate	Derm NCI
	K. H. Kraemer	Clinical Associate	Derm NCI
	P. M. Elias	Clinical Associate	Derm NCI

COOPERATING UNITS (if any)

Surgery Branch, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.15	0

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with severe, recalcitrant, confluent Darier's disease are treated with either dermabrasion or dermatome stripping. Rate of wound healing is measured by histologic examination of sequential punch biopsies taken after (1) incisional wounds, (2) wounds made by 3 mm dermal punches, or (3) sites of dermatome stripped skin. Results indicate that surgical treatment can produce long lasting (over 4 years to date disease free remissions. Furthermore, wound healing, especially epidermal migration, is more rapid in the diseased skin than in uninvolved skin.

Project Description:

Objectives:

1) To observe the response of Darier's Disease to surgical therapy.

2) To study the effect of experimental wounding on the clinical course of both involved and uninvolved skin.

3) To determine if the accelerated healing of dermatome stripped diseased skin in Darier's disease is due to an accelerated epidermal migration.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study of the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

Normal and diseased skin in patients with Darier's Disease.

Methods Employed:

1) Dermatome Stripping in patient 1. Strips of skin were taken from a 62-year-old female patient with Darier's disease using a Castroviejo opthalmologic keratome (Storz Instrument Co.). Strips were approximately 2 cm. in width, 5 cm. in length and 0.2 mm. thick, determined by using a 0.2 mm. spacer in the shaving head. The wounds were then left open, measured and photographed daily.

Incisional Wound Healing. Multiple linear incisions, approximately one cm. in length, were made in diseased skin areas of the right anterior forearm and in clinically uninvolved skin of the left anterior forearm. Four mm. punch biopsies were taken at zero time, and 3, 6, 12, 18, 24, 48, and 72 hours after wounding.

2) Dermabrasion Therapy in patient 2. A 26-year-old man with hypertrophic conifying Darier's disease especially severe on arms and legs was treated with surgical dermabrasion under general anesthesia. Rate of wound healing was measured by serial 6 mm punch biopsies of circular defects produced by 3 mm punches, and by serial punch biopsies taken from dermatome stripped sites of diseased and uninvolved skin punches.

3) Dermatome Stripping in patients 3 to 5. Two women and one man with severe, confluent, inflammatory, vesicular Darier's disease were treated with dermatome stripping of legs or buttocks under general anesthesia. Rate of wound healing was measured by the methods used in

patient 2. One of these women also had dermabrasion of the face and excision of severely diseased labia majora.

Major Findings:

1) Dermatome Stripping in patient 1. Involved sites healed more rapidly than uninvolved skin following dermatome stripping. Two weeks after stripping, the uninvolved site remained larger and more erythematous than the involved site. Furthermore, a zone approximately 10 cm. wide of previously diseased skin around stripped sites on the left hip was observed to have clinically regressed by this time. Clinical regression of involved skin surrounding dermatome stripped sites was repeatedly observed.

Five months after the initial stripping experiment, the patient had a generalized exacerbation of her disease, which spared previously dermatomed sites of uninvolved skin. Histologically, the epidermis and pilosebaceous apparatus appear normal except for diminution of the rete ridge pattern and flattening of the papillary dermis.

Incisional Wound Healing. Superficial linear incisions healed more quickly in diseased sites (12 hours) than in uninvolved sites (48 hours) indicating accelerated epidermal migration.

2) Diseased sites healed more rapidly than uninvolved sites, using both methods of measuring rate of wound healing. Incisional wound healing studies were not done because of the difficulty in locating incisions in diseased tissue after 1-2 days. 3 mm defects in diseased skin healed in 2 d, and in uninvolved skin in 3 d. The patient responded well to dermabrasion and had less than 10% recurrence after 11 months of observation. Peripheral clearing, as seen in patient 1, was not observed.

3) Dermatome stripping was well tolerated in all patients producing long lasting (over 4 years) disease free remissions. Peripheral clearing has occurred in 3 of 5 patients following dermatome stripping.

Significance to Cancer Research:

1) Knowledge of the controls governing cell migration after injury are essential to the understanding of malignant growth.

Proposed Course of Project:

1) Continued long-term observation of treated patients.

2) Confirmation of these findings in other patients with Darier's disease.

Publications:

1) Dellon, A.L., Peck, G.L., Chretien, P.B.: Hypopharyngeal and laryngeal involvement with Darier's Disease. Arch. Dermatol. 111: 744-746, 1975.

2) Dellon, A.L., Chretien, P.B., Peck, G.L.: The epidermectomy procedure for Darier's Disease. A motion picture presented at the American Academy of Dermatology, December 1975, San Francisco, Calif., and the Conference on the Biochemistry of Epidermal Differentiation, July 1975, Ann Arbor, Michigan. The film will be made available for purchase and rental from the Institute for Dermatological Communication and Education, San Francisco, California.

3) Peck, G.L., Kraemer, K.H., Wetzel, B., Klingler, W.G., Cohen, I.K.: Cornifying Darier's Disease - A unique variant. I-Case Report, Arch. Dermatol. 112:495-503, April 1976.

4) Cohen, I.K., Kraemer, K.H., Peck, G.L.: Cornifying Darier's Disease-A unique variant. II. Surgical Treatment. Arch. Dermatol. 112: 504-506, April 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3644-05 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

A Screening System for Topical Chemotherapy of Mycosis Fungoides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. L. Peck Senior Investigator Derm NCI

COOPERATING UNITS (if any)

Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI
Laboratory of Toxicology, DCT, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Contracts are currently being awarded to establish a program to topically screen chemotherapeutic agents for possible therapeutic use in mycosis fungoides, and subsequently in benign hyperplastic diseases, such as psoriasis, and in cutaneous carcinoma.

After chemotherapeutic agents have been supplied by the DCT, NCI, and after these agents have been screened for percutaneous absorption and skin irritation in experimental models, then clinical testing will be done initially in mycosis fungoides.

PHS-6040
(12-75)

Project Description:

Objectives:

The establishment of a contract program in which present and future chemotherapeutic agents may be screened topically for antimitotic potential, toxicity, and possible therapeutic use initially in the malignant skin disease, mycosis fungoides, and subsequently in benign hyperplastic diseases such as psoriasis and in cutaneous carcinoma.

The Objective and Approach of the National Cancer Plan that this project closely supports is 6.2: Discover new therapeutic agents and develop means for their selection.

Patients Needed:

At least 5 adult men or women for each chemotherapeutic agent will be tested topically in the treatment of mycosis fungoides.

Methods Employed:

After chemotherapeutic agents have been supplied by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, and after these agents have been screened for skin irritation and percutaneous absorption, clinical testing will be done in mycosis fungoides. The initial clinical testing will be done using patch test screening for biological activity.

The clinical protocols of this large scale clinical testing will be formulated by the prime contractor. NCI is currently negotiating with Johns Hopkins University to perform the clinical testing by contract. Hopkins heads a cooperative group studying mycosis fungoides. The preclinical testing will be done by the University of Mississippi School of Pharmacy on a subcontract from Batelle Corp., prime contractors to the Laboratory of Toxicology, DCT, NCI.

Significance to Cancer Research:

1) This program is a national cooperative study designed to discover superior topical chemotherapeutic agents than those which are currently available in the treatment of skin cancer, mycosis fungoides, and psoriasis.

2) We would also hope to contribute to the knowledge of the clinical pharmacology of these agents. Skin cancer and mycosis fungoides should prove valuable for the screening of topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients with skin cancer can be expected to be in good

health, which allows for long term studies.

Proposed Course of Project:

The entire design of this screening system, including preclinical screens and large scale clinical testing, is currently being established with the assistance of a committee of dermatologists, pharmacologists, toxicologists, and chemotherapists. This program will complement an Arthritis Institute program for psoriasis.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3632-06 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Toxic Epidermal Necrolysis and Graft-Versus-Host Reaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Peck	Senior Investigator	Derm NCI
OTHER	P. M. Elias	Clinical Associate	Derm NCI
	T. J. Lawley	Clinical Associate	Derm NCI
	G. P. Herzig	Hematology Supportive Care Branch, NCI	
	R. G. Graw, Jr.	Hematology Supportive Care Branch, NCI	

COOPERATING UNITS (if any)

Hematology Supportive Care Branch, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	0

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical study of patients with Graft-Versus-Host Reaction (GVH) has led to the discovery of one patient whose cutaneous eruption progressed to Toxic Epidermal Necrolysis - Histological and ultrastructural studies are being done on this patient and on current patients with GVH.

PHS-6040
(12-75)

Project Description:

Objectives:

To study Graft-Versus-Host Reaction (GVH) and Toxic Epidermal Necrolysis (TEN), and to investigate the relationship between them.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Methods Employed:

1) Examination of a patient with acute lymphocytic leukemia, treated with allogeneic bone marrow transplantation, who developed GVH, and subsequently TEN.

2) Examination with the electron microscope of multiple skin biopsies taken at various time intervals and at various skin sites.

Major Findings:

1) Clinical Findings: A case was observed in which the usual morbilliform eruption of GVH was associated with sheet-like desquamation of the epidermis, typical of TEN. Farly in the clinical course of this patient, cutaneous histopathology was typical for GVH. Later in the clinical course, biopsies taken from intensely erythematous sites, near areas of massive epidermal desquamation showed more extensive epidermal destruction, consistent with the diagnosis of TEN. Support for our thesis linking TEN in this patient with his GVH comes from animal studies in which experimental GVH in hamsters and monkeys developed TEN.

2) Electron microscopic findings: In biopsies taken at distances greater than 10 cm. from the blister edge, ultrastructural changes were limited to the basal cell layer. The entire basal layer was involved in a uniform manner, while the spinous, granular, and horny layers appear virtually uninvolved. Mitochondrial swelling, coarsening of tonofibrils and increased prominence of ribosomes were noted in all basal cells. The nuclei appeared contracted, with more convoluted nuclear membranes: increased density of chromatin was noted. Biopsies closer to the blister edge displayed considerable intercellular edema and occasional early bulla formation. Lymphocytes were occasionally seen within the basal layer, but only rarely above. Increased numbers of Odland bodies and abnormal keratohyalin granules were noted in the superficial epidermis. In summary, the initial pathologic events are in the basal layer, and it appears that the mononuclear infiltrate may play a significant pathogenetic role.

Significance to Cancer Research:

1) Immunosuppressed patients, including treated cancer patients, who are transfused or transplanted with immunocompetent cells, may expect to develop GVH. In view of this case report, we may now expect some of them to develop TEN as well.

Proposed Course of Project:

Electron microscopic examination is continuing. Specimens from new patients with either TEN or GVH are also being studied.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3638-07 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of DNA Repair in Normal Human Cells and in Cells from Xeroderma Pigmentosum Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. H. Robbins	Senior Investigator	Derm NCI
OTHER:	A. D. Andrews	Clinical Associate	Derm NCI
	L. B. Lyons	Expert Consultant	Derm NCI
	F. W. Yoder	Clinical Associate	Derm NCI
	W. C. Cheng	Visiting Fellow	MO NCI
	J. Whang-Peng	Senior Investigator	MO NCI
	S. B. Zimmerman	Senior Investigator	LMB A
	R. S. Day III	Research Physical Scientist	CH NCI

COOPERATING UNITS (if any)

Medicine Branch, Chemistry Branch, Lab. of Molecular Biology, NIAMD

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.3	2.7

SUMMARY OF WORK (200 words or less - underline keywords)

Studies in this laboratory are designed to elucidate the role of DNA repair processes in human disease, especially in human carcinogenesis and ageing. Most studies have been conducted with cells from patients with xeroderma pigmentosum (XP), all of whom have defective DNA repair plus the clinical abnormalities of premature ageing of the skin and cutaneous malignancies. We measure the DNA excision repair process by assays of UV-induced unscheduled DNA synthesis and are using these assays to study the UV dose response of the excision system and the effect of in vitro ageing on this system. We assess the biological effectiveness of DNA repair with assays of post-irradiation colony-forming ability and have found a striking correlation between the level of effectiveness of DNA repair in XP and the severity of XP-associated neurological abnormalities. We are studying the relationship of UV-induced chromosome abnormalities and of UV-induced mutations to the repair defects of XP. We are extending our work to include cells from patients with other diseases in which DNA repair defects are known or suspected. We have established long-term lymphoblast lines from all six genetic forms of XP which will be a useful tool, especially in biochemical work on the molecular identification of the repair defect.

Project Description:

Objectives:

To study DNA repair processes in normal cells, cells from patients with each of the six genetic forms of xeroderma pigmentosum (XP), and cells from patients with other diseases in which defective DNA repair is known or suspected; to determine the relationships between the function of such DNA repair processes and the clinical findings of photosensitivity, premature ageing, and skin or other cancers.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Materials:

Cells are obtained from hospitalized patients and outpatients at the NIH, and occasionally as gifts from other investigators. Cells currently under study include dermal fibroblasts, peripheral lymphocytes, and long-term lymphoblast lines.

Methods Employed:

Rates of excisional DNA repair are determined by using scintillation spectroscopy and autoradiography to measure the unscheduled incorporation of tritiated thymidine into X-ray or ultraviolet (UV)-irradiated cells. The biological effectiveness of repair is tested by assays of post-irradiation colony-forming ability (CFA). Mutability of cells to UV or X-ray is tested in assays which select for clones of cells resistant to oubain or deficient in HGPRT.

Major Findings:

1) Autoradiographic studies of XP cells after UV irradiation have shown that a very small (ca, 1% of normal) but detectable amount of UV-induced unscheduled DNA synthesis occurs in cells from patients in complementation group A, the genetic form of XP having the least such synthesis. This work has demonstrated the great sensitivity of our assay system; no other laboratory has been able to measure such small amounts of unscheduled DNA synthesis.

2) Autoradiographic studies of repair are being performed to determine if there are qualitative differences in the dose-response of repair synthesis after UV irradiation among XP fibroblast strains.

3) Autoradiographic screening of cells for the presence of mycoplasma (detected as cytoplasmic incorporation of tritiated thymidine) has

been shown to be of great value, especially when dealing with those species of mycoplasma not easily detected by standard culturing techniques. Such screening is of great importance to all work on DNA repair as mycoplasma contamination can significantly alter the results of repair assays and if undetected will lead to erroneous conclusions.

4) Autoradiographic studies are underway to determine the effects of in vitro ageing of fibroblasts on the DNA excision repair system. Such studies will compare the rates of UV-induced unscheduled DNA synthesis at different passages of certain XP and normal fibroblast strains.

5) We have studied post-UV colony-forming ability (CFA) in XP fibroblasts from each of the six genetic forms of XP. A striking correlation between the degree of reduction in post-UV CFA and the presence and severity of XP-associated neurological abnormalities has been found. These results constitute the first strong evidence for a relationship between the effective level of DNA repair and the severity of a heredo-degenerative condition of the nervous system. These results support the theory of "intrinsic mutagenesis" which hypothesizes that error-proneness in key DNA repair or replication enzymes is ultimately responsible for a variety of degenerative diseases as well as for normal ageing in various human tissues and for carcinogenesis, especially in the case of those cancers whose incidence increases with age.

6) The post-UV CFA studies of certain XP strains have revealed a somewhat greater UV resistance at high UV doses than at low UV doses. Cloning experiments are in progress to determine if such results are due to the presence of two distinct cell populations or are a reflection of dose-dependent differences in repair efficiency intrinsic to all the cells in these strains.

7) We have confirmed recent brief reports that cells from patients with Cockayne's syndrome, a syndrome consisting of cachectic dwarfism, neurological abnormalities and photosensitivity, have decreased post-UV CFA. We have studied cells from six unrelated patients and are establishing additional new strains. The studies have already revealed that there are significant differences among Cockayne strains in the degree of reduction in post-UV CFA. No defects in UV-induced unscheduled DNA synthesis have been found, however, in any of these strains. Despite this, the probability that these cells do have DNA repair defects is high, as one patient in our XP series was found to have the unmistakable clinical findings of Cockayne's syndrome in addition to the signs of XP.

8) Studies in co-operation with Dr. Rufus Day, III, of the NCI are being conducted on the Cockayne cells to determine if these cells have reduced repair of UV-irradiated adenovirus 2. Such a reduction if found, would clearly indicate the existence of DNA repair defects in this syndrome.

9) Studies in co-operation with Dr. Steven Zimmerman have shown that the levels of DNA ligase activity are normal in Cockayne's cells as well as in XP.

10) Studies in our lab have confirmed reports of decreased post-X-ray CFA in cells from patients with ataxia telangiectasia, a disease in which patients have an increased rate of cancer, plus degenerative findings in the immune and nervous systems. Such studies are now being performed in parallel with post-UV CFA assays on XP cells, Cockayne cells, and cells from patients with other photosensitive and or degenerative disorders.

11) We have succeeded in establishing long-term lymphoblast lines from at least one representative of each of the six genetic forms of XP. Work to date with several such lines indicates that they retain the same level of diminished DNA repair as the fresh peripheral lymphocytes from which they arose. Further characterizations of all such lines is underway. These lines should be valuable in attempts to characterize and purify the defective DNA repair enzymes in XP. Efforts are being made to establish such lines from patients with Cockayne's syndrome and ataxia telangiectasia as well.

12) A clinical study of patients with Cockayne's syndrome is in progress. Complete work-ups of all abnormal clinical findings in such studies should give us an unparalled opportunity to relate the clinical manifestations of this syndrome to the laboratory findings, as has been and is being done with XP patients.

13) In collaboration with Dr. Weng-Chow Cheng and Dr. Jacqueling Whang Peng of the NCI, studies of UV-induced chromosome abnormalities and sister-chromatid exchanges are being performed using normal and XP lymphoblast lines. It is already clear that UV-induced gross chromosomal abnormalities and sister chromatid exchanges are greater in most XP cells than in normals. Comparisons between the different genetic forms of XP are now being made.

14) A major study of UV mutability of normal and XP fibroblasts is now underway. Two different mutant selection systems are being utilized: Oubain resistance and 6-thiogianine resistance (HGPRT deficiency). These studies will compare mutability among all six forms of XP and will then be extended to include other diseases of interest, such as Cockayne's syndrome.

15) Attempts are being made to accomplish <u>in vitro</u> malignant transformation of XP and normal fibroblasts by UV light. If successful, the results of such work when correlated with our knowledge of DNA repair efficiency and UV mutability should yield important new information concerning the interrelationships of DNA repair, mutagenesis and carcinogenesis.

Significance to Biomedical Research and the Program of the Institute:

UV-radiation is believed to be the major cause of the most common type of human cancer, cancer of the skin. Patients with XP are particularly susceptible to the carcinogenic action of UV-radiation and develop multiple malignancies on sun-exposed areas of skin. Normal human cells have an excisional repair process which rapidly repairs DNA damage while most XP patients have a marked impairment in the rate of such repair. This process is involved not only in repair of UV-induced DNA damage but also in repair of damage by certain chemical carcinogens. Understanding the relationship between DNA repair deficiency and skin tumor develop in XP patients would, therefore, elucidate the role of DNA repair in preventing in other humans those cancers which may be due to certain chemical and physical carcinogens. Extension of this work to include study of other diseases in which DNA repair may be defective, especially those associated with an unusually high incidence of cancer such as ataxia telangiectasia will of course further increase our understanding of the relationships between DNA repair processes and carcinogenesis.

Proposed Course of Project

Continuation of research as indicated in the foregoing.

Publications:

1) Newsome, D.A., Kraemer, K.H., and Robbins, J.H.: Repair of DNA in xeroderma pigmentosum conjunctiva. Archives of Ophthalmology 94: 660-662, 1975.

2) Robbins, J.H., Kraemer, K.H., and Andrews, A.D.: Inherited DNA defects in H. sapiens: Their relation to UV-associated processes in xeroderma pigmentosum in Biology of Radiation Carcinogenesis (ed. by J.M. Yuhas, R.W. Tennant, and J.B. Regan), Raven Press, New York, 1976, pp 115-127.

3) Kraemer, K.H., DeWeerd-Kastelein, E.A., Robbins, J.H., Keijzer, W., Barrett, S.F., Petinga, R.A., and Bootsma, D.: Five complementation groups in xeroderma pigmentosum. Mutation Research 33:327-340, 1975.

4) Kraemer, K.H., Andrews, A.D., Barrett, S.F., and Robbins, J.H.: Colony-forming ability of ultraviolet-irradiated xeroderma pigmentosum fibroblasts from different DNA repair complementation groups. Biochimica. et Biophysica Acta. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 701 CBD 3647-03 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Scanning and Transmission Electron Microscopy of Leukocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. K. Wetzel	Senior Investigator Derm NCI
OTHER:	L. Weiss	Professor of Anatomy, Dept. of Anatomy, Johns Hopkins University School of Medicine
	Li-Tsun Chen	Assistant Professor of Anatomy, Dept. of Anatomy, Johns Hopkins University School of Medicine

COOPERATING UNITS (if any)

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.25	1.25

SUMMARY OF WORK (200 words or less - underline keywords)

To define the surface ultrastructure of leukocytes under a wide range of conditions and thereby provide fresh insight into the physiology of normal and leukemic cells; also, to characterize definitively the origin, contents and fate of the characteristic granules of neutrophils, eosinophils, and basophils as an index of the development, function and fate of these cells.

PHS-6040
(12-75)

Project Description:

Objectives:

To define the surface ultrastructure of leukocytes under a wide range of conditions and thereby provide fresh insight into the physiology of normal and leukemic cells; also, to characterize definitively the origin, contents, and fate of the characteristic granules of neutrophils, eosinophils, and basophils as an index of the development, function and fate of these cells.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

Normal human peripheral blood, passing directly from a vein through a large needle into a ten-fold volume of warm swirling fixative, has been prepared for SEM in order to evaluate the influence of conventional isolation procedures on leukocyte topography.

Methods for retaining fixed leukocytes on a suitable substrate in high yields have been explored and we are currently using several strongly polar compounds to adhere the cells on Millipore filters.

Following perfusion with fixative (2% glutaraldehyde in 0.1M phosphate buffer), thymic lymphocytes have been studied in situ by fracturing samples frozen in ethanol, or by mechanically disrupting the tissue after critical point drying.

Major Findings:

The most direct fixation of normal human peripheral leukocytes reveals a remarkable similarity among them with regard to their surface morphology. Nearly all lymphocytes, monocytes, and granulocytes appear spherical and covered with uniform short microvilli. Occasional discrete ruffles occur on each cell type. When fixed in suspension in conventional buffy coat preparations or in samples from Ficoll-Hypaque gradient centrifugation, the neutrophils are changed very little, (perhaps with fewer microvilli), but very few lymphocytes retain discrete ruffles, and most monocytes become covered with ridges or ruffles. If lymphocytes are collected on silver filters prior to fixation, as recommended by others, their topography is drastically altered; when collected from Hank's balanced salt solution the majority of normal peripheral lymphocytes become smooth-surfaced, but if 10% fetal calf serum is present, the microvilli on most lymphocytes become exaggerated. These observations account for much of the variation among published accounts of lymphocyte surface form.

Thymocytes are generally smooth-surfaced when viewed in situ. The vast majority of circulating lymphocytes are thought to originate (or exist for a significant period) in the thymus. Thus, they must develop microvilli, since virtually all circulating lymphocytes appear villous.

Significance to Cancer Research:

The reactions of lymphocytes and macrophages to immune responses in malignant growth and therapy are largely dependent on cell surface phenomena and cell interaction -- both uniquely amenable to study by SEM and ancillary methods.

A thorough understanding of the origin and nature of leukocyte granules is necessary to define and interpret the differentiation of normal granulocytes, the cytopathology of chronic granulocytic leukemia and related disorders, and to help clarify the nature and functions of granulocytic infiltrates in skin and other tissues in malignant diseases.

Proposed Course of Project:

We are engaged in a thorough description of the topography of peripheral leukocytes from normal humans obtained in representative yields. This approach will be extended to studies of hematopoiesis and the leukemias. A major study of leukocyte granulogenesis is planned employing quantitative autoradiography, cytochemistry and TEM. Light microscopic monitoring of preparative conditions and the dynamics of leukocyte adherence, motility, interaction, and growth will be continued with the use of thin perfusion chambers.

Publications:

1) Alexander, E., and Wetzel, B.: Human lymphocytes: Similarity of B and T cell surface morphology. Science 188: 732-734, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3648-04 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

T Cell Leukemias and Lymphomas: Morphologic and Functional Studies of the Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Lutzner	Senior Investigator	Derm NCI
OTHER:	R. L. Edelson	Clinical Associate	Derm NCI
	P. Schein	Georgetown University Hospital	
	I. Green	Medical Officer, Research	I LI
	C. Kirkpatrick	Senior Investigator	I LCI
	T. Ahmed	Bethesda Naval Hospital, Research Unit	
	M. Facktor	Bethesda Naval Hospital, Research Unit	

COOPERATING UNITS (if any)

National Institute of Allergy and Infectious Diseases
Bethesda Naval Hospital, Research Unit

LAB/BRANCH

Dermatology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

To characterize the structure and function of the abnormal cells in the T cell leukemias.

Precise identification of inflammatory cells in lesions.

To study structure, function and oncological significance of the characteristic cell of the Sezary syndrome, and to study similar parameters in malignant cells of mycosis fungoides and related cancers. (1) Patients sought with Sezary syndrome and with mycosis fungoides. Specimens of blood, marrow, skin node, etc. will be obtained for study: (2) Tissue cells and buffy coat preparations will be Feulgen-stained and examined with a cytophotometer in order to detect presence of polyploid cells; (3) Tissue biopsies and buffy coat pellets will be examined with the electron microscope for morphologic identification of characteristic cells: (4) Chromosome analysis of abnormal tissue, marrow and blood cells will be made in order to determine if chromosomes are normal or abnormal in number and structure; and (5) Immunologic techniques will be used to determine whether Sezary cells belong to either B-or T-lymphocyte class.

Frozen sections of tissue are layered with sheep erythrocytes containing on their surfaces moieties which will specifically interact with receptors on mononuclear cells. Histiocytes have receptor for IgG antibody and B cells have receptors for the third component of complement.

Project Description:

Objectives

1) To determine whether those neoplastic cells which preferentially infiltrate the skin share common features.

2) To relate the clinical findings in these patients to the distinctive properties of the neoplastic cells.

3) To establish more effective means of therapy of these disorders through exploitation of the distinctive properties of the neoplastic cells.

4) To use the homogenous cells found in these disorders as reagents for investigation of T cell function.

5) To isolate the product of the mixed leukocyte reaction locus.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

1) Tissues to be studied are sectioned in a cryostat, layered with sheep erythrocytes containing on their surface moieties, which will specifically interact with receptors on mononuclear cells, rinsed to remove nonadherent cells, fixed in glutaraldehyde, and stained with H and E to be viewed by light microscopy.

2) Tissue, cells and buffy coat preparations are Feulgen-stained and examined with cytophotometer in order to detect presence of polyploid cells.

3) Lymphocytes are grown in short term culture and the karyotype determined.

4) Lymphocytes and tissue were fixed and stained routinely for electron microscopy.

5) Lymphocytes are tested for responsiveness to the mitogens phytohemagglutinin, pokeweed and concanavalin A and for stimulating and responding capabilities in mixed leukocyte cultures.

6) Lymphocytes are tested for sheep erythrocyte receptors, complement receptors, Fc receptors, IgG receptors, surface immunoglobulin, and specific antigenic determinants.

7) Lymphocytes, fibroblasts and keratinocytes are tested for effector and target cell potential in cell mediated lympholysis reaction.

8) An antiserum specific for T cells has been produced and purified by absorption with B cells, red cells, and platelets. This antiserum may be used therapeutically in cases unresponsive to other more conservative measures.

9) Leukapheresis, a process by which blood is passed through a continuous flow centrifuge with white cells being selectively removed from the buffy coat, has been used in the therapy of one patient.

Major Findings:

1) T cells, of at least certain morphologic varieties, preferentially infiltrate the skin and spare the bone marrow.

2) If the Sezary syndrome is redefined as T-cell leukemia with associated erythroderma, we have now identified three morphologic variants.

3) Each patient in this expanded Sezary group differs from the other patients at the cellular level, using functional, antigenic and morphologic criteria. In addition, several patients had unique clinical features, i.e. Jin's inverse cyclicity and F.S.'s recurrent hepatic and renal infiltration.

4) The first case of lymphocytic leukemia with both B and T cell markers on the same cells was identified.

5) Review of the cumulative NIH experience with the Sezary syndrome revealed a remarkable lack of thymic infiltration at autopsy. This plus the identification of T cells in the skin of our more recent patients suggests that the skin may actually be playing a role in the aberrant differentiation of these abnormal cells.

6) An affinity of T cells for the skin is supported by our observation that the large majority of cells in mycosis fungoides, lichen planus, Mucha Habermann's disease, parapsoriasis en plaque and lymphomatoid papulosis also appear to be T cells.

7) One patient with T cell leukemia and tumor stage mycosis fungoides superimposed on a generalized erythroderma was treated with leukapheresis, a process by which leukocytes are selectively removed from the buffy coat in a continuous flow centrifuge, and the cutaneous lesions resolved. The patient returned 100 days later from California for a repeat of the leukapheresis with similar results.

8) Cells from one patient with the Sezary syndrome have been maintained

in culture for one month and retain both their receptors for sheep erythrocytes and their distinctive morphology.

9) An immunotherapy protocol has been devised and accepted for use. However, because of the relative success of our more conservative therapeutic approaches, immunotherapy has not yet been required. Other approaches include the present trial run of L-asparaginase which appears to be of at least limited efficacy.

10) Three patients with cutaneous T cell leukemia have now been treated by leukapheresis. One patient, who presented at the time of initial therapy, with tumor stage mycosis fungoides superimposed on a generalized erythroderma and with a lymphocytosis of more than 200,000 per cu mm has been adequately managed for the last 18 months by leukapheresis alone. Two other patients have also significantly benefited from leukapheresis. One patient is presently undergoing extracorporeal irradiation to determine whether this is a more efficient means of managing patients with lower peripheral blood lymphocyte counts.

11) Cells from one patient were hypotonically lysed following leukapheresis and membrane components were conjugated to a cyanogen bromide activated sephadex column. This column was subsequently used as an immuno-absorbent column to remove and then concentrate the anti-T cell activity and the active factors are themselves valuable reagents.

12) Serum from each of 6 tested patients with cutaneous T cell leukemia contained large amounts of macrophage inhibitory factor, which could be traced back to the leukemia cells. The serum of these patients therefore represent reservoirs from which this material can be isolated and characterized. Of clinical significance, the presence in the serum of a factor which has a profound effect on circulating monocytes may be at least in part responsible for observed anergy in the patients and their susceptibility to infection.

13) Patients with lymphomatoid papulosis were evaluated with regard to their clinical presentation, microscopic, ultrastructural and cell surface marker characteristics of their cutaneous infiltrates, their response to challenge with intradermal antigens, and the response of their peripheral leukocytes to mitogen and antigen stimulation.

Preliminary findings revealed that the infiltrating cells of lymphomatoid papulosis were thymus-derived lymphocytes and the nuclear irregularity of the cell of the cutaneous infiltrates was significantly greater than that of infiltrating lymphocytes of delayer hypersensitity skin reactions, with a pattern similar to that seen in the circulating lymphocytes of the Sezary syndrome.

Patients with lymphomatoid papulosis demonstrated normal reactivity to intradermally applied antigen and normal in vitro stimulation of lympho

cytes by mitogens.

Chromosome studies were carried out on 11 NIH patients who had the diagnosis of Sezary syndrome. Heteroploidy, multiple markers including minute and ring chromosomes, and a lack of modality and clone formation, were the common chromosomal findings in this syndrome. Abundant spontaneous division of heteroploid cells in unstimulated peripheral blood cultures, a high percentage of heteroploid cells in stimulated culture, and finally, clone formation, are signs of a fulminant process and lead to the terminal phase of this disease. Early chemotherapeutic eradication of these heteroploid neoplastic cells would therefore be the treatment of choice in this disease.

Significance to Cancer Research:

By identifying the roles of mononuclear cells in defense against non-lymphoid cancer and accurately characterizing the types of lymphoid cells causing various lymphomas, a more rational approach to the treatment and prevention of these diseases is possible.

Proposed Course of Project:

Patient's with this syndrome will be referred to other branches of the Cancer Institute who have become interested in studying Sezary cells and treating Sezary patients.

Publications:

1) Smith, R.W., Blaese, R.M., Hathcock, K.S., Buell, D.N., Edelson, R.L., and Lutzner, M.A.: T and B lymphocyte markers in lymphoid cell research and in human disease. Monograph: Eight Leukocyte Culture Conference, Academic Press, pp. 127-132, 1974.

2) Edelson, R.L., Kirkpatrick, C.H., Shevach, E. M., Schein, P.S., Smith, R.W., Green, I., and Lutzner, M.A.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes: Morphologic and functional studies. Ann. Inter. Med. 80:685-692, 1974.

3) Lutzner, M.A., Edelson, R.L., and Schein, P.: Sezary syndrome (chapter) in Current Dermatologic Management. Published by C. V. Mosby, St. Louis, Missouri, pp. 286-287, 1975.

4) Yoshida, T., Edelson, R., Cohen, S., and Green, I.: Serum macrophage inhibitory factor in patients with T cell leukemia, J. Immunol. 114:914-918, 1975.

5) Gerna-Torsellini, M., Edelson, R.L., Frank, M.M., and Kaplan, A.: Identification of subpopulations of mononuclear cells in cutaneous infiltrates, II. Urticaria. Rolletino Sierroterapica Milanese 54:1, 1975.

6) Ahmed, A., Edelson, R.L., Strong, O., and Green, I.: In vitro reactivity of lymphocytes from patients with chronic T cell leukemia. Clin. Exp. Immunol. (in press).

7) Green, I., Jaffe, E., Shevach, E., Edelson, R., Frank, M., Berard, C.: Determination of the origin of malignant reticular cells by the use of surface markers. Monograph - International Academy of Pathology (in press).

8) Lutzner, M.A., Edelson, R. L., Schein, P., Green, I., Kirkpatrick, C., and Ahmed, A.: Cutaneous T-Cell Lymphomas: The sezary syndrome, mycosis fungoides, and related disorders. Annals of Int. Medicine, Vol. 83: No. 4, 1975.

9) Whang-Peng, J., Lutzner, M., Edelson, R. and Knutsen, T.: Cytogenetic studies and clinical implications in patients with Sezary syndrome. Cancer Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3649-04 D

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Enzymatic Control Mechanisms in Melanogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	V. J. Hearing	Senior Investigator	Derm NCI
OTHER:	J. M. Nicholson	Post-Doctoral Fellow	Derm NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Dermatology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project has partially characterized the nature of tyrosinase, and determined the mechanism of normal latency of the enzyme, and its subsequent activation in vivo. The role of this enzyme in melanogenesis in normal tissue and the importance of the altered enzyme found in melanoma tissues is being studied.

PHS-6040

Project Description:

Objectives:

To investigate the control mechanisms involved in regulating melanogenesis in a variety of normal and malignant tissues.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Tyrosinase and other enzymic activities are investigated both by spectrophotometric and radioactive assay methodology. Tissues are dissected immediately before use in 0.1 M phosphate buffer, homogenized and fractionated into subcellular fractions by means of density gradient centrifugation and differential centrifugation. After solubilization with detergents, enzymes are further purified by polyacrylamide gel electrophoresis. Samples are then incubated with the appropriate substrates and controls. The production of melanin and other reaction products can be followed spectrophotometrically or by liquid scintillation counting of the newly formed radioactive melanin or byproducts.

Major Findings:

Since only one enzyme (tyrosinase) is essential for melanin biosynthesis, it is a unique system for the study of enzymatic control mechanisms in normal and malignant tissues. We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, but not peroxidase, as has been suggested by some workers. It has always been a subject of dispute how tyrosinase, which can be easily demonstrated to be present in an active configuration in the endoplasmic reticulum and Golgi apparatus, is inactivated in vivo in mammalian systems and subsequently activated once in situ in the melanosome. In amphibians, the mechanism seems to be proteolytic activation; in mammalian and avian systems, however, proteolysis has no function in this regard. It has been found in this study that tyrosinase is under allosteric control and that phospholipids may play a part in the expression of the enzyme's activity. It has also been found that this allosteric regulation in murine melanomas is altered; this perhaps explains the atypical melanosome formation in these tissues.

Significance to Cancer Research:

In view of the differences noted between the regulation of tyrosinase in normal and melanoma tissues, we feel that at least one of the primary

differences between the melanogenic capabilities of these two tissues has been revealed. The fact that these controls are operational at the post-translational level is informative; whether other levels of cellular control mechanisms are affected by carcinogenesis remains to be investigated. It is hoped that further study of the cause of these enzymatic differences in the malignant tissues will provide insights into the nature of neoplastic transformation.

Proposed Course of Project:

Differences in the control mechanisms over melanogenesis in normal and malignant melanoma tissues will continue to be investigated. The characteristics of enzymatic activity and control in various subcellular fractions are being studied. This project has been expanded to include characterization of similar metabolisms in normal and malignant human tissues.

Publications:

1) Eppig, J.J.,Jr. and Hearing, V.J.: The bifunctional role of mammalian, avian, and amphibian tyrosinases in melanogenisis. Proc. IX Inter. Pigment Cell Conf. (In press)

2) Hearing, V.J. and Ekel, T.M.: Mammalian tyrosinase: a comparison of tyrosinase hydroxylation and melanin formation. Biochemical Journal (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3650-04 D

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Characterization of Mammalian Melanosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	V.J. Hearing, Jr.	Senior Investigator	Derm NCI
OTHER:	J.M. Nicholson	Post-Doctoral Fellow	Derm NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Dermatology Branch

SECTION

None

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The abnormal proteins produced in malignant melanoma are being studied: these studies are aimed at elucidating the mechanism of formation of these atypical proteins, as well as their importance to the immunology of melanoma and possible immunotherapy potential.

PHS-6040
(12-75)

Project Description:

Objectives:

To characterize the structural and enzymatic composition of melanin granules throughout their development in vivo, both in normal and abnormal pigmentary systems.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Our research has been aimed primarily at characterizing the chemical composition of melanosomes in murine and human pigment systems, both in the normal as well as in melanoma tissues. Tissues are excised and homogenized in 0.25M sucrose, and the melanosomes are then isolated and purified by means of an extensive series of differential centrifugations. Granules thus isolated are solubilized in either sodium decyl sulfate, 8 M urea or Triton X-100, then examined with the aid of polyacrylamide gel electrophoresis. Computer analysis of the resulting protein separations allows the calculation of various physiochemical parameters of these macromolecules, including molecular weight molecular size, valence and several other physical constants that are most useful in the comparisons of various proteins.

Major Findings:

The melanosome in normal mouse tissue is composed of multiple proteins, many of which are loosely bound and easily extracted. These probably constitute the proteins of the limiting membrane of the organelle. Another protein is of lower molecular weight and is tightly bound to the granule, and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. Melanosomal proteins from melanoma tissues vary in structure from those of normal tissue. Several of the proteins seem to be missing completely from these granules, which are also structurally distinguishable from normal granules by electron microscopy. Perhaps more importantly, many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules. This has been found to be the case in human melanoma as well.

Significance to Cancer Research:

The observations concerning the aberrant biochemical characteristics of the melanoma melanosome indicate that the control of melanogenesis in malignant melanoma is in some manner affected by carcinogenesis. Melanomas are unusual among cancers since they do not result in

complete differentiation of the affected melanocyte, but allow this cell's specialization, i.e. melanogenesis, to continue. These results indicate that although melanogenesis takes place, its metabolic pattern is abnormal.

There are a wide range of implications of this research for possible immunotherapy and/or immunoassay of human malignant melanosomes. An analogous situation of altered proteins has been described in several other types of malignancies; thus this process of atypical formation of proteins may be common to neoplastic transformation.

Proposed Course of Project:

Since there are indications that the metabolism of melanogenesis in murine melanoma is aberrant, our research is ultimately intended to examine human melanomas, and attempt to characterize the level on which these disorienting control mechanisms operate. It is hoped that further insight into such controls, be they at the level of replication, transcription, translation, or post-translation, will provide clues as to the level on which carcinogenic information is expressed.

Publications:

1) Klingler, W.G., Montague, P.M. and Hearing, V.J.: Unique melanosomal proteins in murine melanoma. Proc. IX Inter. Conf. Pigment Cell. Biol. (In press)

2) Edelson, R.L., Hearing, V.J., Dellon, A.L., Frank, M., Edelson, E.K., and Green, I.: Differentiation between B cells, T cells, and histiocytes in melanocytic lesions: primary and metastatic melanoma and halo and giant pigmented nevi. Clin. Immunol. Immunopath. 4:557-568, 1975.

3) Hearing, V.J., Klingler, W.G., Edel, T.M. and Montague, P.M.: Molecular weight estimation of Triton X-100 solubilized proteins by polyacrylamide gel electrophoresis. Anal. Biochem. (In press).

4) Klingler, W.G., Montague, P.M., Chretien, P.B. and Hearing, V.J.: Atypical melanosomal proteins in human malignant melanoma. Arch. Derm. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3652-03 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Mechanisms of Cellular Immunity in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. R. Levis Senior Investigator Derm NCI

COOPERATING UNITS (if any)

Reproduction Research Branch, NICHD

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

A better understanding of cell mediated immunity in man. Particularly, normal or abnormal immunologic mechanisms involved in epithelial and lymphoid malignancies, auto-immunity disease, allograft rejection and allergic contact dermatitis. Characterization of the abnormal blastogenic responses found in certain diseases, particularly lymphoid malignancies. The necessity of both lymphocytes and macrophages in blastogenic responses in vitro has been elucidated and the lymphocyte has been identified as the cell which determines the immunologic specificity of the blastogenic response to antigens. A soluble mediator, released by macrophages, has been found to be an essential requirement for the blastogenic response to antigens and foreign tissue. Soluble mediators (lymphokines), released by various subpopulation of lymphocytes, have also been detected and studied at varying times following specifically induced cell-mediated contact sensitivity reactions. Our combined in vitro and in vivo controlled methods for the study of cell-mediated immunity in man, have allowed us to initiate basic immunopharmacology and immunogenetics approaches for the study of a variety of diseases and has already resulted in several practical diagnostic procedures. Furthermore, initial studies offer promise of a predictive index for agents affecting the cellular immune apparatus, which is known to be important in host defense against a variety of neoplastic processes.

Project Description:

Objectives:

1) To investigate the mechanisms concerned with the control of mitosis, differentiation, function, and potentialities of normal and pathologic cells, with particular reference to the lymphocyte and cells of the lymphoreticular system.

2) To investigate the functional and morphological interactions between lymphocytes and macrophages and among different populations of lymphocytes during in vitro immune responses related to delayed hypersensitivity, homograft reactions, and cell mediated allergic contact dermatitis.

3) To detect, characterize, and isolate biologically active substances, including soluble mediators of cellular immunity, produced in stimulated and unstimulated human leukocyte cultures. To study their effects modulation, and differentiation in vitro.

4) To develop an in vitro system for studying allergic contact dermatitis in humans including a method of monitoring immunotherapy of skin cancers with DNCB.

5) To develop a useful and sensitive histocompatibility test in humans.

6) To develop an in vitro model for the study of homograft rejection in humans.

7) To develop an in vitro system for studying immune reactivity to various skin cancers.

8) To determine the efficacy of 2,4 dinitrochlorobenzene (DNCB) on cutaneous malignancies and premalignant growth in subjects sensitized to DNCB.

9) To evaluate DNCB as a useful model for the study of immunotherapy of certain human cancers and possibly lead to additional immunotherapy studies.

10) To develop an in vitro model for studying and screening drugs which modulate cell mediated immunity in humans.

The Objective and Approach of the National Cancer Plan that this project most closely suppors is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (along and in combinations) in the cure and palliation of cancer.

Material:

1) Lymphocytes and other peripheral blood cells are obtained from hospitalized patients and outpatients including patients with chronic lymphocytic leukemia, sezary syndrome, and other disease states.

2) Keratatome strips of human skin.

3) Excised tumors of human skin.

4) Sperm cells obtained from human ejaculates.

5) Fibroblasts, lymphoid cells from humans and other species maintained in tissue culture.

Methods Employed:

1) Human peripheral blood lymphocytes are cultured in vitro and stimulated to transform into large blastoid cells by factors such as phytohemagglutinin, antigens, and foreign tissue. This phenomenon of blastogenesis is evaluated by the morphologic and biochemical changes which the lymphocytes undergo. The mechanisms by which these blastogenic factors act and the culture conditions required for blastogenesis are determined.

2) Human peripheral blood leukocytes are separated into a glass-adherent cell population, composed largely of macrophages, and into a population consisting of small lymphocytes. By recombining such populations obtained from the same and from different individuals, the function of each population is determined. Interactions and contacts between cells are examined by light and scanning electron microscopic techniques, including methods for observing the same cells by both modalities.

3) A method has been developed for freeze storing leukocytes for subsequent studies of the blastogenic response to various antigens and mitogens.

4) A method has been developed for adapting the dinitrochlorobenzene contact sensitization test to the in vitro lymphocyte transformation system.

5) Methods have also been developed for freeze storing human spermatozoa with preservation of HLA-D (LD) antigens.

Major Findings:

1) Methods for obtaining 'purified' lymphocytes, glass-adherent mononuclear cells, and a population containing both cell types from human

peripheral blood have been developed and analyzed. We have improved and utilized a very rapid and simple 5-minute centrifugation method using heparinized whole blood for obtaining a population of mononuclear leukocytes (lymphocytes and monocytes) with very few contaminating granulocytes. This mononuclear leukocyte population contains the two cell types required for the blastogenic response and provides cultures capable of responding with maximal responses to specific and non-specific blastogenic factors.

2) Human epidermal cells induce blastogenesis in allogeneic leukocytes. Human epidermal cells did not induce blastogenesis in autologous leukocytes nor in the leukocytes of a mixed leukocyte HL-A identical sibling pair. These results indicate that epidermal induced leukocyte blastogenesis is a measure of histocompatibility in humans comparable to a one-way mixed leukocyte reaction.

3) A method has been developed which permits positive light microscopic identification of individual leukocytes in a heterogeneous population by Giemsa staining while preserving delicate surface features for subsequent scanning electron microscopy of the same cells.

4) Isolation and partial characterization of a factor released by glass adherent phagocytic cells which is an essential requirement for the blastogenic response of lymphocytes to specific antigens, leukocytes, and phytohemagglutinin.

5) Isolation of a factor released in cultures containing lymphocytes of subjects sensitized to DNCB that will induce blastogenesis in leukocyte cultures from nonsensitized subjects.

6) Characterization of the abnormal blastogenic responses of lymphocytes from patients with chronic lymphocytic leukemia, sezary syndrome, and other related disease states.

7) Development of a method for monitoring the degree of sensitivity to DNCB in vitro during in vivo contact sensitization. This in vitro technique expands the usefulness of DNCB for the evaluation and study of cell mediated immunity, and offers a method for monitoring DNCB immunotherapy, an approach for the study of tolerance, and a controlled system for the study of the many antigen-released mediators of cellular immunity in man. Similar approaches with other contact allergens may offer a safe diagnostic test for allergic contact dermatitis.

8) Successful adaptation of the Dinitrochlorobenzene (DNCB) contact sensitization test to the in vitro lymphocyte transformation technique in humans.

9) Development of simplified methods for freeze storing human peripheral blood leukocytes.

10) Using the frozen leukocyte technology and the method for studying DNCB sensitivity in vitro we have demonstrated a temporal relationship between in vivo skin reactivity and in vitro specific lymphocyte transformation during a primary immune response.

11) Peak in vitro activity was observed two to four weeks following application of the primary sensitizing dose of DNCB and fell off to lower levels within two months of primary sensitization. These sequential studies of allergic contact dermatitis in humans are the first such studies of the kinetics of a primary cellular immune response using both in vivo and in vitro techniques.

12) Additional studies have demonstrated a quantitative relationship between the intensity and duration of allergic contact dermatitis to DNCB in vivo and the magnitude of specific lymphocyte transformation in vitro.

13) Demonstration that repeated challenge with DNCB boosts specific in vitro lymphocyte transformation.

14) Discovery of lymphokine activity in allergic contact dermatitis there is evidence that such lymphokines may be the mediators of cell mediated immunity.

15) Demonstration of the time course of release of lymphokine activity in human peripheral blood cultures.

16) Demonstration of reproducible dose-response curves for lymphokine activity. Such dose-response curves illustrate a sensitive quantitative assay for cellular immunity in vitro and offers a biological system with great potential for further characterization and isolating the mediators in cell mediated immunity.

17) The DNCB system offered the versatility that made it possible to show the lymphokines responsible for lymphocyte transformation in target leukocyte populations from nonsensitized subjects were as active on syngeneic (identical twins) leukocytes as on allogeneic leukocytes, indicating the observed activity was not due to histocompatibility factors.

18) It has recently been shown that lymphokine activity is absent during a primary immune response to DNCB and required one or more rechallenges before activity is detectable using the lymphocyte transformation system which suggests the soluble factors known as lymphokines, may represent an amplification of cellular immunity.

19) Successful adaptation of an automated microsystem for harvesting cells has enabled us to harvest cells in a rapid fashion.

20) The unique features of this project are: a) Both in vitro and

in vivo portions of this project are ethically feasible in humans which makes these studies more germane to human disease. b) Previous studies with microbial antigens have lacked effective control over exposure, in contrast to controlled DNCB sensitization. c) The assay is sensitive, specific and quantitative making it a valuable tool for measuring lymphocyte activation under a variety of experimental conditions.

21) Studies on soluble factors released by human lymphocytes (lymphokines) indicate that little or no lymphokine is released during an in vivo primary immune response to DNCB, but is produced in some subjects only after additional in vivo rechallenge. We interpret this to indicate that the lymphokine activity demonstrated in vitro may well indicate a bodily mechanism for amplifying the cellular immune response.

22) Recent studies in our laboratory indicate that an entire series of DNP containing amino acids induce varying degrees of lymphocyte transformation specifically in subjects topically sensitized with DNCB. The ability to stimulate with soluble DNP containing substances as well as with our previously developed particulate antigen offers great flexibility in our combined in vitro - in vivo system for investigating the cellular immune response in humans. Furthermore, these studies might prove to be of considerable importance in understanding the mechanism of triggering a specific cell mediated immune reaction.

23) Demonstration of the ability of sperm cells to induce allospecific lymphocyte transformation. We have previously demonstrated the ability of macrophages, certain lymphocytes, and human epidermal cells to induce allospecific lymphocyte transformation. Interestingly, studies in the mouse indicated that a product of the major histocompatibility complex has a tissue distribution similar to the cell populations we have found to induce allospecific lymphocyte transformation in humans. These findings could have important impact in diverse areas such as transplantation and reproductive biology. We are currently attempting to find out if our studies on contact sensitivity are related or in any way controlled by the major histocompatibility complex in humans. The combination of our histocompatibility and contact sensitivity studies may develop into a field of human immunogenetics with potential ramifications in a wide variety of disease states.

24) We have developed methods for freeze-storing human spermatozoa and preserving their ability to stimulate allogeneic lymphocytes. This methodology, which uncovers the important HL-A-D (LD) locus responsible for graft rejection allows a practical and superior approach to study of the genetic locus containing immune response genes associated with a variety of human diseases.

Significance to Biomedical Research and the Program of the Institute:

The lymphocyte is a cell involved in immune reactions which may be

operative in the immune response to neoplastic tissue. Our studies concerned with blastogenesis and the control of cell division and differentiation of the lymphocyte may elucidate this cell's normal and abnormal functions in the immune response involved in the host's defense against cancer. These studies should also provide information concerning the control of cell differentiation and division.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Publications:

1) Levis, W.R., Whalen, J.J., and Powell, J.A.: Studies on the contact sensitization of man with simple chemicals III. Quantitative relationships between specific lymphocyte transformation, skin sensitivity and lymphokine activity. J. Invest. Dermatol. 65:100-104, 1975.

2) Powell, J.A., Whalen, J.J., Levis, W.R.: Studies on the contact sensitization of man with simple chemicals IV. Timing of skin reactivity, lymphokine production, and blastogenesis following rechallenge with dinitrochlorobenzene using an automated microassay. J. Invest. Dermatol. 64:357-363, 1975.

3) Levis, W.R., Powell, J.A., and Whalen, J.J.: Hapten vs carrier specificity in contact sensitivity. J. Immunol. 115:1170-1173, 1975.

4) Levis, W.R., Whalen, J.J., and Powell, J.A.: Specific blastogenesis and lymphokine production in DNCB-sensitive human leukocyte cultures stimulated with soluble and particulate DNP containing antigens. Clin. & Exp. Immunol. 23:481-490, 1976.

5) Levis, W.R., Whalen, J.J., and Shevins, R.J.: Mixed sperm/leukocyte cultures as a measure of histocompatibility in man. Science 191:302-304, 1976.

6) Levis, W.R. and Berry, F.: Mechanism of HL-A "Predispositions" to specific diseases. N. Eng. J. Med. 293:1323-1324, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3654-03 D

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Applications of SEM to Soft Biological Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. K. Wetzel	Senior Investigator	Derm NCI
OTHER:	K. Sanford	Head, Cell Physiology & Oncogenesis	LB NCI
	C. Fox	Senior Investigator	LB NCI
	G. Jones	Microbiologist	LB NCI
	S. Roseman	Professor, Dept. of Biology & McCollum-Pratt Inst., Johns Hopkins University	
	B. Walther	Post-Doctoral Fellow, Dept. of Biology, Johns Hopkins University	
	P. Howard	Guest Worker	Derm NCI
	R. Tarone	Staff Fellow	B NCI
	M. Greenstein	Guest Worker	Derm NCI

COOPERATING UNITS (if any)

Laboratory of Biochemistry, Biometry Branch, NCI

LAB/BRANCH
Dermatology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	0.75	2.75

SUMMARY OF WORK (200 words or less - underline keywords)

To discover and develop new methods and interpretive criteria for the application of SEM to a wide variety of soft biological tissues, and thereby capitalize on the unique capabilities of this instrument in providing 1) improved survey and sampling as an adjunct to other studies, 2) an unparalleled three-dimensional, experiential view of cell form and cell surface phenomena, and 3) an unusual potential for cytochemical studies.

PHS-6040
(12-75)

Project Description:

Objectives:

To discover and develop new methods and interpretive criteria for the application of SEM to a wide variety of soft biological tissues, and thereby capitalize on the unique capabilities of this instrument in providing 1) improved survey and sampling as an adjunct to other studies, 2) an unparalleled three-dimensional, experimental view of cell form and surface phenomena, and 3) an unusual potential for cytochemical studies.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.5: Study cell surface and cell membranes.

Methods Employed:

For most studies described herein, tissue culture cells are as monolayers on glass coverslips, fixed by the addition of an equal volume of buffered 6% glutaraldehyde and processed appropriately for SEM. Single cells dissociated from the epidermis of fetal mice were allowed to settle on glass coverslips, grown in primary culture, and sampled periodically for SEM and TEM. Cultures of a spontaneously transformed line of mouse fetal cells of clonal origin were compared with cultures from explants of a tumor produced by cells of that line in the ninth passage. For adhesion studies, 3T12 or 3T3 tissue culture cells in forced suspension, prelabeled by growth in the presence of ^{3}H-leucine, were allowed to settle on monolayer cells of the same type for short periods, then fixed and examined sequentially by SEM and light microscope autoradiography.

Major Findings:

Within one hour after settling on glass coverslips adherent mouse epithelial cells begin to aggregate, and by 12 hours few cells occur individually. Most rounded cells with short microvilli and TEM characteristics of basal cells spread on the coverslip within four hours, assume forms indicative of active locomotion, and aggregate with each other forming desmosomal contacts; other cell types adhere to their upper surfaces. Thus, by 12 hours there was striking organization among the cells with flattened basal cells forming discrete islands against the glass and the other cells clustered on top of them. Between one and seven days in culture increasing numbers of the superficial cells became spherical, smooth-surfaced, and developed pits and perforations indicative of degeneration; TEM of vertical sections through these cell clumps confirmed this supposition.

Among six spontaneously transformed lines of mouse embryo fibroblasts of common single cell origin studied by SEM the cells of one line

appeared remarkably heterogeneous. These cells produced a tumor which was explanted and derivative cells were maintained in culture for nine passages. By SEM the cells of the tumor-derived line were also heterogeneous and the population was indistinguishable from the parent line. Thus, any or all of the many forms in this line may be tumorigenic, and the morphological heterogeneity may be considered a phenotypic trait of this line which persists through growth both in vitro and in vivo.

Isotopically labeled cells from suspension which adhere to monolayers of the same cell type can be unequivocally distinguished from morphologically identical cells in the monolayer by autoradiography. This may be the first successful combination of this technique with SEM. Within two minutes of settling adherent cells show little change from their appearance in suspension; and the initial process of adhesion can be characterized as follows: only a few very small discrete contacts may be involved; they generally occur between processes from the suspension cell and the body of a single monolayer cell; any type of cell process may do -- most suspension cells are villous, but blebs and ruffles when present can evidently accomplish adhesion. On the other hand as the cells spread on the monolayer, marginal ruffles characteristically appear. Within 30 minutes after settling many cells from suspension assume the form of flattened monolayer cells and are unrecognizable except by the label visualised by autoradiography.

Significance to Cancer Research:

SEM reveals fine surface structure with unparalleled three-dimensionality, dept of field, and sampling capability. As this approach is adapted to biological problems through experience with diverse tissues, cellular form, surface details and interactions can be better interpreted in normal and malignant tissues, as well as in experimental systems which help define immune responses, differentiation, and other processes fundamental to oncogenesis and its control. The tissue culture studies directly elucidate new aspects of tumor cell biology and transformation.

Proposed Course of Project:

To further develop and refine techniques of specimen preparation, analysis, and interpretation for SEM will be the goal of continuing work with a wide variety of tissues, experimental approaches, and instrumentation. Special emphasis will be accorded a search for novel cytochemical methods, especially with labels visualized in the backscatter mode, which are uniquely possible for this tool. Further collaboration in SEM, TEM and cytochemical studies of skin is under way with investigators in this branch and elsewhere.

Publications:

1) Blanquet, R.S., and Wetzel, B.: The surface ultrastructure of the

Scyphopolyp, Chrysaora quinquecirrha, as revealed by scanning electron microscopy. Biol. Bull. 148:181-192, 1975.

2) Schaefer, H., and Wetzel, B.: Superior 2x2 slides. G.W. Bailey (Ed.) Proc. Thirty-third Annual Meeting Electron Microscopy Society of America, Las Vegas, Nevada, 1975, pp. 158-159.

3) Wetzel, B., Jones, G., Westbrook, E., Fox, C., and Sanford, K.: Bailey (Ed.), Proc. Thirty-third Annual Meeting Electron Microscopy Society of America, Las Vegas, Nevada, 1975, pp. 516-517.

4) Peck, G.L., Kraemer, K.H., Wetzel, B., Klingler, W.G., and Cohen, I.K.: Cornifying Darier's disease - a unique variant. Arch. Dermatol. 112:495-503, 1976.

5) Wetzel, B.: Cell Kinesics: An interpretative review of the significance of cell surface form. In Scanning Electron Microscopy, 1976 (Johari, Om., Ed.), ITT Research Institute, Chicago, Illinois 135-144 and 702-704.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3656-03 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemistry, Structure and Biosynthesis of Mammalian Epidermal Keratin Filaments

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Steinert	Visiting Associate	Derm NCI
OTHER:	G. L. Peck	Senior Investigator	Derm NCI
	S. Yuspa	Section Head	DCCP NCI

COOPERATING UNITS (if any)

Experimental Pathology Branch, DCCP

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The polypeptide chains which comprise the subunits of the keratin filaments of normal bovine epidermis have been isolated and characterized. The polypeptides polymerize in vitro into filaments which have the same physical and chemical structure as the in situ keratin filaments. The polymerization in vitro follows an ordered biphasic cooperative mechanism and analysis of the details of this process will provide information on the ultrastructure of the epidermal keratin filament.

The polypeptide subunits obtained from abnormal human epidermis are different from those of normal epidermis, implying the existance of abnormalities in the chemistry and structure of the proteins. Further work is in progress to identify such differences.

Normal mouse epidermal cells grown in cell culture to study the biosynthesis of the keratin proteins do not synthesize keratin. Rather, the cells synthesize large amounts of a muscle actin-like protein. Interestingly, a variety of human and mouse epidermal tumors also contain large amounts of the actin-like protein. The possibility of using the actin as a biochemical marker in vivo and in cell culture for studies on malignant epidermal tumor is being investigated.

PHS-6040
(12-75)

Project Description:

Objectives:

1) To characterize the structure of epidermal keratin filaments isolated from normal bovine epidermis.

2) To investigate the mechanism of assembly of the isolated polypeptide chain subunits of the keratin filaments into filaments *in vitro*.

3) To characterize the polypeptide subunits of keratin filaments isolated from various benign and malignant human epidermal diseases.

4) To investigate the biosynthesis of the keratin filament polypeptide subunits in cultures of normal mouse epidermal cells and to investigate the effects of various drugs and carcinogens on the cells.

5) To characterize the actin-like protein detected and isolated from normal mouse epidermal cells grown in culture and from mouse and human epidermal tumors. An attempt will be made to develop a specific immunofluorescent- or radio-immune-assay for the actin-like protein.

Major Findings and Methods Employed:

1) The seven polypeptide chains which comprise the subunits of the keratin filaments of normal bovine epidermis have been isolated and individually characterized by standard protein chemical techniques. The chains can be classified into three distinct groups on the basis of close similarities in such properties as α-helix contents, molecular weights, amino acid compositions and N-terminal amino acid residues.

2) The isolated polypeptides spontaneously polymerize *in vitro* in dilute salt solution into filaments that are uniformally 80 Å wide and up to 40 μm long. The polypeptide composition of these filaments, and their structure, based on electron microscopy and X-ray diffraction, are the same as the *in situ* keratin filaments.

3) Mixtures of the purified chains were prepared and it was found that many combinations of as few as two chains formed filaments *in vitro* in high yield. By electron microscopy these filaments have the same structure as those assembled from all seven chains. Interestingly, the combinations resulted in filaments containing one mole of one polypeptide and two moles of a second polypeptide, which is suggestive of the formation of a triple-chained unit, and this is supported by X-ray diffraction analyses. In all cases, one polypeptide from at least two of the above mentioned groups was necessary for *in vitro* filament assembly.

4) Studies on the mechanism of filament assembly indicate that polymerization is time and protein concentration dependent and follows an ordered biphasic cooperative process. This involves the initial rate-limiting formation of a "nuclear" particle followed by a rapid aggregation of it into filaments. The nuclear particle is a hexamer, as determined by mathematical models of light-scattering data, and has dimensions of 40Å X 1000Å. Filament formation appears in the electron microscope to involve (a) apparent twisting of two or three aligned 40Å units to form an 80Å particle; and (b) end-to-end joining of either the 40Å units or 80Å particles. More extensive studies on these events are in progress.

5) The α-helical segments in the filaments polymerized in vitro from two polypeptides will be isolated to examine such questions as the alignment and staggering of the polypeptides in the filament. Initial sequence studies are planned by isolation and ordering of CNBr peptides.

6) Comparisons of the polyacrylamide-gel electrophoretic profiles of the keratin subunits obtained from abnormal human epidermis such as Darier's disease and lamellar ichthyosis with those of normal epidermis show prominent differences in numbers and mobilities of bands. Also, the abnormal polypeptides do not polymerize into filaments in vitro. Therefore, there may be differences in the chemical structures of the proteins. Attempts are underway to identify such differences by comparisons of two-dimensional gel electrophoretic maps of the CNBr peptides of the proteins obtained from normal and abnormal epidermis.

7) Mouse epidermal cells grown in culture do not synthesize the normal complement of keratin polypeptides, and the proteins produced do not polymerize in vitro into keratin filaments. Many different culture conditions have been explored to restore this, but none has been successful. However, the cells under all conditions tested synthesize large amounts of an actin-like protein which is probably present in the cells as microfilaments. The protein has been isolated and is being characterized.

8) A variety of human and experimentally-induced mouse epidermal tumors (carcinomas and papillomas) also contain large amounts of the actin-like protein. Because this observation has great potential as a marker for malignancies, the actin protein is being isolated and attempts will be made to prepare an antibody for a specific immunofluorescent- or radio-immuno-assay procedure. The large amounts of actin protein should provide a suitable biochemical marker for studies of the malignancies. The presence of actin protein in mouse epidermal cell cultures may also provide a marker for studies on in vitro carcinogenesis.

Significance to Cancer Research:

The epidermis offers a unique opportunity for the study of tumors not

only because of the quantitative importance of this tissue but also because of its accessibility. One of the major problems in studying malignancies of the epidermis has been the lack of a suitable biochemical marker. The keratin filaments are the most prominent intracellular components of all epidermal cells and therefore a study of their chemistry, structure and biosynthesis in both normal and abnormal epidermis will be of profound importance in studying tumors in this tissue. The finding of large amounts of actin protein in epidermal tumors, and the development of suitable immunoassay procedures for it is of potentially great significance.

Proposed Course of Project:

Studies on the diseased abnormal, benign and malignant tissues will be done in collaboration with Dr. Gary Peck. The cell culture studies will be done in the Experimental Pathology Branch in collaboration with Dr. Stuart Yuspa. The biochemical, biophysical and electron microscopic studies in the polymerization of normal bovine epidermal keratin filaments will be done in this laboratory.

Publications:

1) Steinert, P.M.: The extraction and characterization of Bovine epidermal α-keratin. Biochem. J. 149:39-48, 1975.

2) Steinert, P.M.: The heterogeneity of bovine epidermal α-keratin. J. Invest. Dermatol. 64: 210, 1975.

3) Steinert, P.M. and Idler, W.W.: The polypeptide composition of bovine epidermal α-keratin. Biochem. J. 151:603-614, 1975.

4) Steinert, P.M., Peck, G.L., Yuspa, S.H., DiTasquale, A., and McGuire, S.: The isolation of an actin-like protein from epidermal tumors and cultured epidermal cells. J. Invest Dermatol. In the press.

5) Steinert, P.M. and Gullino, M.I.: Bovine epidermal keratin filament assembly in vitro. Biochem. Biophys. Res. Commun. In the press.

6) Steinert, P.M., Idler, W.W. and Zimmerman, S.B.: The polypeptide composition of bovine epidermal keratin filaments polymerized in vitro. Fed. Proc. Fed. Amer. Soc. Exptl. Biol. In the Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3657-02 D

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunopathologic Mechanisms Involved in Blistering Skin Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.I. Katz	Senior Investigator	Derm NCI
OTHER:	K.C. Hertz	Clinical Associate	Derm NCI
	H. Yaoita	Expert Consultant	Derm NCI
	T.J. Lawley	Clinical Associate	Derm NCI

COOPERATING UNITS (if any)

Metabolism Branch, NCI

LAB/BRANCH

Dermatology Branch

SECTION

None

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Pemphigus, herpes gestationis, bullous pemphigoid and dermatitis herpetiformis are blistering skin diseases all of which are strongly associated with so-called autoimmune phenomena. The studies in this laboratory have been mainly concerned with the identification and characterization of serum and in vivo bound antibodies in the latter 3 diseases. Our major investigations have centered around the mechanisms of tissue destruction, ultrastructure, and ultrastructural localization of these antibodies. Gastrointestinal abnormalities, which are very similar to those seen in gluten sensitive enteropathy occur in dermatitis herpetiformis and are also under active study. Immunogenetic considerations with regard to HLA associations and the identification of specific B-lymphocyte antigens have provided us with the tools required to study the triggering mechanisms involved in the early stages of an abnormal immune response. Another major aspect of our work is the study of the effect of sulfones on neutrophilic exudative disorders. As sulfones dramatically alleviate dermatitis herpetiformis and erythema elavatum diutinum we are using animal models of inflamation and serum and blister fluids from patients to study their effect on complement activation, deposition and neutrophilic chemotaxis.

Project Description:

Objectives:

1) To investigate the mechanisms involved in the expression of certain immunologic skin diseases, namely pemphigus, bullous pemphigoid, dermatitis herpetiformis and herpes gestationis.

2) To determine the cell surface characteristics of the cells involved in these diseases.

3) To determine whether or not genetic factors are important in the developments of these diseases.

4) To determine the ultrastructure and ultrastructural localization of antibodies in blistering skin diseases.

Material:

Skin biopsies, either punch of Castroviejo keratome slices will be used. Also the small intestine of patients with dermatitis herpetiformis will be studied in order to determine their binding characteristics. Blister fluid studies for inflammatory mediators are also under investigation.

Methods Employed:

Direct and Indirect Immunofluorescence Cell Mediated Cytotoxicity, Immunochemical methods for identifying immunoglobulins and immune complexes. Radioimmunoassays.

Major Findings:

1) Almost all of 40 patients with dermatitis herpetiformis have in vivo bound IgA and various complement components.

2) The exact location of these antibodies has been sought and found to localize ultrastructurally in 1) clumps in the dermis, 2) associated with anchoring fibrils in the dermis or 3) along the lamina lucida of the basement membrane.

3) The early ultrastructural destruction of the skin in these patients has been demonstrated to correlate well with the antibody localization.

4) Almost all patients with dermatitis herpetiformis have morphologic and enzymatic evidence of gastrointestinal abnormalities very similar to those seen in patients with gluten-sensitive enteropathy.

5) In some patients it is possible to not only reverse the gastrointestinal abnormalities but to reduce or completely eliminate their

requirement for sulfones by strict adherence to a gluten-free diet.

6) 85% of 39 patients with immunologically proven dermatitis herpetiformis have HLA-B8 as compared to 20% of controls.

7) All patients with dermatitis herpetiformis have a specific antigen on their B-lymphocytes. This antigen is not present in the normal population.

8) A 50% potassium iodide occlusive patch test in most cases produced a lesion histologically identical to de novo lesions in dermatitis herpetiformis.

9) Sulfones dramatically benefit patients with erythema elevatum diutinum.

10) By studying five patients with herpes gestationis with immunologic methods we have been able to identify the serum factor responsible for depositing complement at the skin basement membrane zone, the site of earliest destruction.

11) Ultrastructural localization of complement correlates well with the earliest destruction seen in the electron microscope in herpes gestationis and further strengthens the concept that immunologic mechanisms play an important, if not essential, role in the pathophysiology of this disease.

Significance to Cancer Research:

A basic understanding of immunologic injury in various autoimmune disease states is important in interpreting and further current concepts in self-recognition. Pemphigus is associated with thymoma and myasthemia gravis and its study may provide a clue as to the association between pemphigus and other malignancies. Our in depth studies of herpes gestationis, an immunologic blistering disease of pregnancy, may provide important clues to maternal-fetel interactions.

Proposed Course of Project:

Outlined above.

Publications:

1) Mann, D.L., Katz, S.I., Nelson, D.L., Abelson, L. ., Strober, W.: Specific B-cell antigens associations with gluten-sensitive enteropathy and dermatitis herpetiformis. Lancet 1:110, 1976.

2) Katz, S.I., Hertz, K.C., Yaoita, H.: Herpes gestationis. Immunopathology and characterization of the HG factor. J. Clinical Invest. (In press)

3) Hsueh, A.J.W., Dufan, M.L., Katz, S.I., Catt, K.J.: Gonadotropin receptors. Immunofluorescent labeling in enzyme dispersed interstitial cells. Nature (In press).

4) Yaoita, H., Gullino, M., Katz, S.I.: Herpes gestationis ultrastructural localization of in vivo bound C3 and ultrastructure. J. Invest Derm. (In press).

5) Katz, S.L., Hertz, K.C., Rogentine, G. N., Strober, W.: The association between HLA-B8 and dermatitis herpetiformis in patients with IgA deposits in skin. Archives of Dermatology (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3658-02 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Search for Animal Models for Genetic Skin Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Lutzner	Chief	Derm NCI
OTHER:	R. L. Kent	Biologist	Derm NCI
	K. Smith	Geneticist	R VR
	C. T. Hansen	Geneticist	R VR

COOPERATING UNITS (if any)

Division of Research Services, Veterinary Resources Branch

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

It has been discovered that the vibrissaeless rat blisters because of a suprabasilar adhesion defect; that the exfoliative mouse suffers a transient purulent conjunctivitis, exfoliates and may die. Survivors continue to have intermittant hair loss; masked rats have blepharitis accompanied by alopecia. Pasteurella organisms appear to be playing a role. The motheaten mouse has been found to have a hyperimmunoglobulinemia and usually dies of an autoimmune disease. Germfree environment has no effect on prolonging survival. An attempt has been made to classify and summarize genodermatoses involving neoplasia.

PHS-6040
(12-75)

Project Description:

Objectives:

To seek out animal models for human genetic skin diseases. Such mutations have occurred spontaneously or been produced in various animal production laboratories; some in our own animal laboratories at NIH.

Materials:

1) The Vibrissaeless (vb) rat, an autosomal recessive mutation, produced by multiple generation X-irradiation.

2) A mutation in the mouse occurring spontaneously called (me) which appears to have an immunological abnormality manifested by runt syndrome and early death.

3) A mouse mutant, which loses its hair in peculiar pattern and develops an inflammatory disease of the eyes.

4) A rat mutant in which the animals' eyes and snout are involved in an inflammatory disease.

5) A mutant mouse, in which the immunologic, endocrinologic and neurologic system are all involved.

Methods Employed:

Animals are bred either by heterozygote or homozygote matings. Some of the animals are kept in germ-free conditions, especially those that present immunologic defects. Tissues from these animals are studied by light and electron microscopy, by immunofluorescence, and by chemical and microbiological techniques.

Major Findings:

1) In the vibrissaeless rat it has been found that animals lose their hair at an early age and develop both spontaneous and traumatic blisters. Blisters can be produced by rubbing the skin. Histologically, separation occurs above the basal cell layer; therefore this is a suprabasalar blistering disease. Although there are several human diseases which resemble this defect, the rat does not appear to be an exact model for any known human genetic disease. Immunofluorescent studies have shown that this is not similar to pemphigus; collagen and collagenase studies have been normal; and animals have been found not to respond to systemic steroid therapy. The biochemical defect which is occurring in these rats has not been uncovered as yet.

2) The me mouse: Since these animals die at a very early age, detailed

immunologic studies have been impossible. Some of the animals have small thymuses and absent Pyer's patches. It has been found that unlike the nude mouse whose survival improves in a germfree environment this environment has no effect on the me mouse's survival.

3) The ex mouse: A spontaneous autosomal recessive mutation has been named the exfoliative mouse (genotype ex/ex). Exfoliative mice suffer a transient purulent conjunctivitis in their 3rd week and an exfoliative skin disease in their 4th week. Gram-negative bacilli are present in blood and cerebrospinal fluid during the conjunctivitis stage, and in skin during the exfoliative period. The mutant, whose exfoliative skin resembles the skin in human lamellar ichthyosis, can be differentiated from the ichthyotic mouse mutant.

4) The Masked rat: Two organisms have been isolated from the urine and eyes of these rats. They are both pasturella organisms and Koch's postulates have been satisfied in showing that these organisms are etiologically involved in producing the scaling inflammatory disease which occurs on the eyes and the snouts. The genetic basis of the susceptibility to these organisms has not yet been determined.

5) The Lethargic mouse: This is a mouse which has neurologic, endocrinological and immunologic abnormalities. No explanation has yet been found.

Significance to Biomedical Research and the Program of the Institute:

Many genetic diseases of the skin occur in which there is no knowledge of cause or treatment. These diseases are rare and experimentation is not easily performed on humans; therefore, it is of interest to try to develop or discover animal models for these diseases in the hope that when such animals have been found it will be possible to find the cause and treatment for these genetic abnormalities which would be hopefully translatable to human disease.

Proposed Course of Project:

Continuation of research as indicated.

Publications:

1) Lutzner, M.A., and Hansen, C.T.: Skin blisters and hair loss in a rat mutant called Vibrissaeless. J. Invest. Dermatol. 65:212-216, 1975.

2) Kent, R.L., Lutzner, M.A. and Hansen, C.T.: Masked rat: An x-ray induced mutant with chronic blepharitis, alopecia and pasteurellosis. J. Heredity, 1976 (In press).

3) Kent, R.L., Lutzner, M.A. and Smith, K.P.: Skin exfoliation and purulent conjunctivitis in a new mutant - the exfoliative mouse. J. Invest. Dermatol., 1976 (In press).

4) Lutzner, M.A., Hansen, C.T.: Motheaten: An immunodeficient mouse with markedly less ability to survive than the nude mouse in a germ-free environment. J. Immunol., 1976 (In press).

5) Lutzner, M.A.: Monograph: The genetics of Human cancer. Edited by John Mulvihill, National Cancer Institute, 1976. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CBD 3660-01 D

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Transfection of Viral DNA of C-Type RNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. R. Lowy Senior Investigator Derm NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

A sensitive, quantitative assay is being developed to study the biologic effects of the DNA viral intermediate and provirus of mammalian C-type RNA viruses. This procedure will be used to assess the ability of DNA to be infectious and/or to induce cellular transformation. Optimal conditions for extraction and titration of viral-specific DNA are being defined. The effect of chemical and physical treatment on virus expression will be evaluated. Differences between unexpressed and expressed endogenous and exogenous viral genomes are under study. Preliminary results indicate that DNA from AKR mouse cells (which contain endogenous viral genomes) can be rendered infectious when cells are treated with 5-iododeoxyuridine prior to extraction of DNA, while DNA from untreated cells is not infectious. The physical changes in DNA which underly these biologic differences are being explored.

Project Description:

Objectives:

1) To develop a sensitive quantitative biologic assay of viral-specific DNA (transfection) from C-type viruses.

2) To determine the effect of a variety of physical and chemical treatments of the transfecting ability of viral-specific DNA.

3) To determine the physical differences between unexpressed and expressed endogenous DNA, since transfection of expressed viral DNA is more easily accomplished than that of unexpressed viral DNA.

4) To define the smallest size of cellular DNA which can yield infectious virus.

5) To investigate the mechanism of activation of endogenous C-type viruses by halogenated pyrimidines.

6) To determine if transfection by the DNA viral intermediate and/or the DNA provirus is sensitive to the murine cellular Fv-1 gene, which restricts the replication of some classes of murine C-type viruses.

7) To define that the portion of the sarcoma virus genome which induces cellular transformation.

8) To utilize transfection as a method to detect portions of viral genomes in tumors, including those of human origin.

Methods:

1) For the isolation of provial genomes, DNA is extracted from tissue culture cells or tumors by the Marmur technique, except that proteinase K is used instead of pronase, since higher molecular weight DNA is obtained by this modification.

2) For isolation of the DNA viral intermediate, DNA is purified by the Hirt procedure.

3) Transfection of DNA utilizes the calcium phosphate technique of Graham and Van der Eb.

4) Virus is grown in sensitive tissue culture cells and purified by sucrose density centrifugation in a zonal rotor. Isotopically labeled single stranded viral DNA probes are synthesized in an endogenous reverse transcriptase reaction carried out in the presence of actinomycin D.

5) Nucleic acid hybridization between viral probes and cell DNA is carried out in liquid and analyzed either by S_1 nuclease or hydroxyapatite chromatography.

Major Findings:

1) Substitution of proteinase K for pronase in the purification of DNA from mouse cells results in DNA with an average molecular weight of greater than $50X10^6$ Daltons.

2) Transfection is readily accomplished with DNA from productively infected cells, but not that from uninfected cells, even when these are known to contain multiple, but unexpressed complete viral genomes.

3) After 24 hours of treatment with 5-iododeoxeine, cellular DNA from cells of the ARR mouse will transfect the viral genome after cells have been treated with 5-iododexyridine for 24 hours. DNA from untreated cells will not transfect.

Significance to Cancer Research:

1) C-type viruses have been implicated in tumors of a wide variety of species, including man. Correlation of biologic and biochemical aspects of viral specific DNA will be of considerable help in elucidating the physiology of mammalian C-type viruses.

2) Since ease of activation of endogenous C-type viruses by IUdR appears to correlate with their case of spontaneous activation, elucidation of the mechanism of activation would probably have relevance to spontaneous viral activation and perhaps to chemical carcinogenesis as well.

3) Transfection represents a technique which may be able to detect biologic activity of portions of viral genomes. Since some tumors in man may be induced by replication of defective viral genomes, this approach may be suitable for their detection.

Proposed Course of Project:

Continuation with attempted fulfillment of stated objectives.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3661-01 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Human Papilloma Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. R. Lowy Senior Investigator Derm NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

A combined biologic, biochemical, and serologic approach is being utilized to study the papilloma viruses associated with human warts. A radioimmune assay for viral antigen has been developed and is being refined. Purification of viral DNA will be accomplished for nucleic acid hybridization studies. These procedures will be used to monitor efforts to propagate virus in tissue culture as well as to evaluate the relatedness of virus in various types of warts. The relationship to other papova viruses will be explored. Skin from a variety of disorders of epidermal proliferation, including cancers associated with epidermodysplasia verruciformis, will be evaluated for the presence and expression of papilloma virus genetic material. Other malignant tissue will be studied virologically.

Project Description:

Objectives:

1) To develop a radioimmune assay for wart viral protein(s).

2) To develop a nucleic acid hybridization procedure for wart viral DNA.

3) To propagate wart viruses in tissue culture.

4) To measure the relatedness of viruses of warts from different patients, including those from patients with epidermo displasia verruciformis (EDV), laryngeal papillomas, condyloma accuminata, flat warts, common warts, plantar warts, and warts associated with immuno-deficiency.

5) To look for wart virus specific nucleic acid and/or antigen in skin cancers of EDV, kerato-acathomas, and other tumors.

6) Relatedness of wart virus to other papova viruses.

Methods:

1) Purification of wart viruses from warts by CsCl density gradient centrifugation.

2) Isotopically labelling viral antigen and viral DNA with ^{125}I for conducting radioimmune assay and nucleic acid hybridization studies.

3) Purification of viral antigen by SDS gel electrophoresis and alkaline degredation.

4) Raise antibodies to wart virus in rabbits.

5) Isolation of viral DNA by ethidium bromide cesian chloride density, gradient centrifugation.

Results:

1) Milligran quantities of full virus particles can be purified from warts. Electron micrographs and SDS gels indicate these preparations are quite pure.

2) Viral proteins can be labeled with ^{125}I and separated by SDS gel electrophoresis.

3) Antiserum to virus reacts specifically with purified virus in double diffusion gels.

4) A radioimmune assay for viral antigen has been developed and is

being refined.

Significance to Cancer Research:

Warts represent a viral induced tumor of man. A closely related virus, the Shope papilloma virus of rabbits, will induce benign tumors in some rabbits of malignant tumor in others. The squamous cell cancers of patients with EDV may be induced by wart viruses, as may tumors of other patients. The development of these reagents will not only permit the screening of tumors for wart viral material, they will also facilitate attempts to propagate the virus in tissue culture. Assuming that the analogy with the Shope papilloma virus is correct, propagation of the virus in tissue culture would facilitate the investigation of the molecular mechanisms which result in a benign proliferation of cells in some instances and malignant transformation in others.

Proposed Course of Project:

Continuation with attempted fulfillment of stated objectives.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 3662-01 D

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Factors Involved in the Elimination of Superficial Mycotic Infection in Guinea Pigs.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Lutzner	Senior Investigator	Derm NCI
OTHER:	A. D. Hernandez	Clinical Associate	Derm NCI
	J. E. Bennett	Senior Investigator	NIAID

COOPERATING UNITS (if any)

National Institute of Arthritis and Infectious Diseases

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

A sensitive assay of fungal growth is being developed to study the possible factors involved in the limitation and elimination of superficial mycotic infections in guinea pigs, with emphasis being placed on possible role of cellular immunity and non-specific serum inhibitors.

PHS-6040
(12-75)

Project Description:

Objectives:

1) Quantitation of fungal spore germination using radiolabeled precursors.

2) Evaluate possible factors involved in limitation and elimination of superficial mycotic infection

a) epidermal turnover rate
b) delayed hypersensitivity and lymphokines
c) serum inhibition factors
d) non-specific products of inflammation

Methods:

1) Radiolabel defined culture media used to grow spores which are germinating - counts are correlated with visual quantitation of increase in size of hyphal element.

2) Guinea pigs will be infected with T. Mentagrephytes and course of infection monitored via skin biopsy and skin test antigens.

3) Sera from guinea pigs infected with T. Mentagrephytes and those in addition challenged with BCG will be studied for inhibition against T. Mentagrephytes and other fungi.

4) Lymphokines from guinea pigs stimulated by specific and non-specific antigen (Ag) will be assessed as to their ability to inhibit fungal growth.

5) Study effects of increasing epidermal proliferation on elimination of infection.

Major Findings:

In progress

Significance to Cancer Research:

Patients with immunosuppression (natural or induced) are susceptible to malignancy, as well as to bacterial, fungal and viral infections. Study of immune system from the point of view of mycotic infections, might provide insight into mechanisms involved and suggest treatment for such infections.

Publications:

None

ANNUAL REPORT OF THE METABOLISM BRANCH
SUMMARY OF SIGNIFICANT ACTIVITIES
NATIONAL CANCER INSTITUTE

July 1, 1975 through June 30, 1976

The clinical research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, a broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasms as well as in experimental animal systems to determine the nature of the immune processes that are of importance in the surveillance against neoplastic cells and to define the nature of defects in the immunological system that result in an increased incidence of neoplasia. Major efforts in this area are also directed toward developing techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy. The second major goal of the Branch is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Both patients with neoplastic disease as well as those with non-neoplastic disorders that facilitate the development of techniques for the study of cell membranes, homeostatic mechanisms and metabolic derrangements of these control mechanisms are being investigated.

Detection of Circulating Tumor Associated Antigens

Sensitive quantitative radioimmunoassays for the oncofetal proteins alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG-β) were established in previous years and were shown to be of value in the diagnosis of certain forms of malignancy in that elevated values were demonstrated in a significant number of patients with cancer, but in virtually no patients with benign disorders. The radioimmunoassays for AFP and hCG were evaluated as adjuncts to the diagnosis of testicular malignancy and in monitoring the effectiveness of therapy of this disorder. Ninety percent of 200 patients with germinal neoplasms of the testis had an elevated value of one or both of the marker proteins. Thrity-three patients with a testicular mass were studied with the markers prior to surgery. Both markers were negative in the 29 patients who were ultimatly shown to have no germinal tumor of the testis. In contrast, 5 of the 8 patients with a germinal tumor had an elevated serum level of alpha-fetoprotein or hCG. Thus, the use of markers appears to be of value as an adjunct to the diagnosis of cancer of the testis. The radioimmunoassays for these markers were of even greater value in monitoring the effectiveness of therapy of the testicular tumor. The effect of therapy on the clinical course and on the serum levels of AFP and hCG was evaluated in 33 patients who had elevations of both markers prior to therapy. In 9% of the cases there was no clinical response to chemotherapy and the levels of both markers remained stable or increased. In 15% of the cases the marker levels dropped to and remained at the normal level. These patients have had a complete disappearance of their disease with no recurrence. In 30% of the cases the markers declined with moderately effective therapy but both markers remained above the normal range and increased when the tumor recurred clinically. In 33% of the cases,

both markers declined but one of the markers remained above the normal range and the remaining marker fell to normal levels. With recurrence of the tumor one of the markers remained at the normal level whereas the other returned to a very high level. In one patient representing 3% of the series, the tumor markers fell following chemotherapy but the tumor mass failed to diminish. At surgery this patient was shown to have a mature teratoma following therapy with loss of the malignant elements. In 33% of the cases evaluated there was an elevation of one or both of the markers at times following therapy when there was no clinical evidence of residual disease. In each of these cases there was a recurrence of clinically evident tumor within the subsequent year. Thus, it is apparent that all elevated values of AFP or hCG in patients with germ cell tumors reflect residual tumor. With this in mind, it is our view that these markers should be used in protocol studies in which patients with testicular tumors are treated. The presence of a positive marker should be taken into consideration in determining the future course of the patient's therapy. The apparent discordant behavior of the tumor markers in 33% of the patients in which one but not both markers fell to normal levels following therapy and remained normal following recurrence of the tumor suggested that the cells of origin of these markers were probably different. This conclusion, was confirmed in studies performed in conjunction with Dr. Robert Kerman. In these studies an indirect immunoperoxidase test using monospecific rabbit anti-alpha-fetoprotein and monospecific rabbit anti-human chorionic gonadotropin were used to define the cell of origin of these markers. AFP was demonstrated in mononuclear "embryonal" cells within both embryonal carcinoma and entodermal sinus tumor components. hCG in contrast was identified within syncytiotrophoblastic cells in both embryonal carcinoma and choriocarcinoma elements. Thus, these markers had different cell types of origin. In all cases where serum levels of both markers were measured at the time of tissue acquisition there was a complete correlation between the markers present in the tissue sections and the markers elevated in the serum.

Immunophysiology and Clinical Immunology

A significant effort of the Metabolism Branch over the past years has been directed toward defining the nature of the events of cellular differentiation, cellular interaction and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens and to define the pathological defects in patients with a primary immunodeficiency state or with a malignancy of the T- or B-lymphocyte system. The first of these studies was directed at defining the factors controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo a sequential maturation process from stem cells originating in the marrow to B-lymphocytes. Then following interaction with antigen or mitogen the B-cells mature into immunoglobulin synthesizing plasma cells. The majority of patients with primary immunodeficiency diseases as well as patients with chronic lymphocytic leukemia appear to have a defect in the terminal differentiation of B-lymphocytes into immunoglobulin synthesizing and secreting cells. Previously we developed techniques for the study of the terminal differentiation of such B-lymphocytes. More recently we have focused on the role of regulatory T-cells in the control of this maturational event. In these studies of regulatory T-cells we have used a technique we have developed to define excessive numbers of suppressor T-cells as well as two new

techniques we have developed this year, one to detect and quantitate helper T-cells in man and the second to define deficiency in negative regulatory T-cells. When these techniques were applied to the study of patients with common variable hypogammaglobulinemia the patients were shown to be heterogenous with a variety of different immunological defects. Some of the patients had a circulating inhibitor of B-cell maturation; a significant number of patients had intrinsic defects of the B-cells and a significant subset of patients with common variable hypogammaglobulinemia had an abnormality of regulatory, suppressor T-cells which act to inhibit B-cell maturation and antibody synthesis. This latter subset of patients did not appear to have an intrinsic defect in the maturation of their B-lymphocytes but rather had an abnormal number or abnormally activated set of suppressor T-cells. Patients with thymoma and associated hypogammaglobulinemia were also shown to have excessive numbers of activated suppressor T-cells which may play a role in the hypogammaglobulinemia observed in these patients.

The nature of the defect in patients with isolated IgA deficiency was then investigated using the in vitro technique to study pokeweed mitogen driven terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. The cultured lymphocytes from each of the 14 patients with isolated IgA deficiency studied synthesized normal quantities of IgG and IgM but secreted no IgA into the media. However, 11 of the 14 patients studied synthesized IgA by the 7th day in pokeweed mitogen stimulated cultures as assessed by staining for cytoplasmic IgA using fluorescine labeled anti-IgA antisera. These individuals had normal numbers of IgA specific helper cells and did not have any circulating suppressor cells. Thus, these patients with isolated IgA deficiency can synthesize IgA but appear to have a defect in IgA secretion. A second group of patients involving 3 of the 14 patients did not have lymphocytes with IgA demonstrable in their cytoplasm following culture. When the lymphocytes from these 3 patients were co-cultured with normal lymphocytes and pokeweed mitogen the synthesis of IgA by the normal cells was depressed by 80-100%. Synthesis of IgG and IgM was not depressed. Thus, this smaller group of patients with isolated IgA deficiency did not synthesize IgA and had IgA specific suppressor cells that prevent B-cells from maturing into IgA synthesizing and secreting cells.

Further studies of the immunoregulatory role of T-cells have been pursued in an experimental animal model of humoral immunodeficiency. In avian species, a central lymphoid organ, the Bursa of Fabricius, is the site of initial differentiation of B-lymphocytes. Removal of the Bursa in the new hatched period results in agammaglobulinemia in the birds that survive. This agammaglobulinemic state is characterized by total deficiency of B-lymphocytes and plasma cells, absence of germinal centers in the lymphoid tissue of these birds, and a total absence of serum IgM, IgA, and IgG. Cellular immune function, however, is preserved. T-cells from agammaglobulinemic chickens were found to have profound effects on humoral immune responses when transplanted into normal birds. Such T-cell graft recipients also developed agammaglobulinemia despite the continued presence of a Bursa of Fabricius in these birds. This "infectious agammaglobulinemia" was characterized by total absence of IgM, IgA and IgG but differed from that induced by bursectomy by the persistence of B-lymphocytes and germinal centers in the lymphoid tissues. Thus, the immune defect in these T-cell graft recipients closely parallels the

defect observed in many patients with common variable hypogammaglobulinemia who also have a persistence of B-lymphocytes and evidence for excessive "suppressor T-cell" activity.

Further studies of the nature of this phenomenon have shown that suppressor T-cells develop in bursectomized birds after all Ig has disappeared from their circulation and that such suppressor T-cells have specificity for each of the immunoglobulin classes. Thus, when limiting members of T-cells from bursectomized agammaglobulinemic birds are transplanted into normal recipients, some recipient birds develop deficiency of all classes of Ig while others develop dysgammaglobulinemia. This dysgammaglobulinemia is characterized by loss of one or more, but not all, classes of Ig. T-cells obtained from these dysgammaglobulinemic birds are then capable of transferring this class specific suppression to other normal birds but do not suppress Ig classes which were normal in the donor chicken. Thus, animals with selective deficiency of IgG, or IgA, or IgM as well as birds with deficiency of IgG and IgA, or IgM and IgA, or IgM and IgG have been produced. This Ig class specific suppression also closely parallels various human immune deficiency diseases with deficiency in one or more classes of Ig and provides an excellent experimental model to further investigate the role of T-cell regulation of Ig production and potential therapeutic maneuvers to correct such deficiencies in man.

As discussed above excessive numbers of suppressor T-cells may be associated with hypogammaglobulinemia. However, it should be noted that such cells normally play a key role in the negative control of immune system in general and in the development of specific immunological tolerance. We have recently developed evidence from an animal model system of autoimmunity that suggests that loss of precursors of suppressor T-cells can lead to autoimmune diseases. In these studies we have used NZB/W mice that have a genetically determined autoimmune disorder similar to that observed in patients with systemic lupus erythematosis. Abnormalities in these mice include defects in the induction and maintenance of tolerance to foreign antigens, the development of antibodies with specificity for nucleic acids, erythrocytes and lymphoid cells, the development of immune complex glomerulonephritis, a Coomb's positive hemolytic anemia and a high incidence of reticuloendothelial neoplasms. To approach the question of T-cell suppressor function in NZB/W mice more directly we have taken advantage of the observation that splenic T-cells can be activated by Concanavalin A (Con A) to become cells which suppress the immunological functions of other cells. Spleen cells from normal mice as well as from young and adult NZB/W mice cultured in the presence of pokeweed mitogen synthesized 860 ± 120 ng IgM/10^6 cells/7 days. Concanavalin A (Con A 2 µg/cc) added to the culture caused a 94% suppression of IgM synthesis in adult normal mice and in young NZB/W mice but did not suppress IgM synthesis in adult NZB/W mice. To analyze this defect we studied the suppressor activity of Con A pulsed cells. Spleen cells from normal mice cultured with Con A for 24 hours washed free of Con A produced an 88% suppression of IgM synthesis when cocultured with normal spleen cells in the presence of pokeweed mitogen. Supernatants of normal spleen cells pulsed with Con A for 24 hours contain a soluble immune response suppressor (SIRS). Spleen cells from both young and adult NZB/W mice could respond to suppressor signals in that they were suppressed by Con A pulsed normal spleen cells or supernates therefrom. When NZB/W mice were studied in the presence of suppressor cells we found that young NZB/W

Con A pulsed spleen cells caused suppression of IgM synthesis by spleen cells from a variety of sources whereas adult NZB/W Con A pulsed cells lacked such suppressor activity. We conclude that NZB/W mice lose suppressor T-cell activity as they age and suggest this loss of suppressor cells may be a significant pathogenetic mechanism in the development of autoimmunity in these animals. The demonstration in this study that a soluble immune response to suppressor (SIRS) derived from T-cells following Con A stimulation exists and the observation that NZB/W mice are responsive to this negative signal raised the possibility of treating these mice with SIRS. Indeed, in preliminary studies, the administration of SIRS to the NZB/W mice from an early age led to the development of hypogammaglobulinemia in these animals as well as a virtual prevention of the development of anti-DNA antibodies, Coomb's positive hemolytic anemia, and immune complex nephritis. The occurrance of an abnormality in suppressor T-cell function in NZB/W mice suggests that a similar phenomenon may be characteristic of human systemic lupus erythematosis and other human conditions associated with autoimmunity and further suggests the possibility of treating humans with these disorders in a more physiological manner with the soluble immune response suppressor protein.

The tests of in vitro immunoglobulin synthesis and helper and suppressor function were applied to the lymphocytes of patients with a malignancy of the T- or B-cell system. The malignant cells of patients with chronic lymphocytic leukemia are B-lymphocytes with IgM and IgD immunoglobulins on their surface. The chronic lymphocytic leukemia cells could not perform helper T-cell functions and did not act as suppressors in co-culture with normal cells. When cultured in vitro these lymphocytes had reduced synthesis of all immunoglobulin classes. However, they preferentially produced significant quantities of IgM when cultured in the presence of pokeweed mitogen. Thus, these cells appear blocked at a stage in development before the production of IgA and IgG molecules. However, the IgM bearing leukemic lymphocytes in these patients were not completely blocked in the sense that they retained the capacity to differentiate after pharmacological stimulation into IgM synthesizing and secreting cells. The peripheral blood leukemic cells in patients with the Sézary syndrome have a T-cell derivation. The peripheral blood T-cells of normal individuals represent a variety of different types of T-cells that cannot be separated from one another. The monoclonal production of T-cells of a single type in the Sézary syndrome, thus, provides the great opportunity of defining the function of an isolated class of T-cells. The neoplastic lymphocytes from patients with a Sézary syndrome did not synthesize detectable immunoglobulins, in addition, they did not have suppressor cell capacity. However, the lymphocytes from 3 of the 4 patients with the Sézary syndrome studied were shown to have a marked capacity to function as helper T-cells. They appeared to represent a monoclonal expansion of the helper T-cell population.

Patients with multiple myeloma have been shown to have a significant reduction in the serum levels of polyclonal immunoglobulins and in the capacity to synthesize antibody in response to antigenic challenge. When the in vitro immunoglobulin biosynthesis test was applied to the peripheral blood lymphocytes from patients with myeloma they showed profound depression of immunoglobulin synthesis to approximately 10% of the normal mean values. Peripheral blood lymphocytes from the majority of patients with myeloma tested suppressed

the polyclonal immunoglobulin synthesis of normal lymphocytes when co-cultured with them. However, in contrast to the observations in patients with common variable hypogammaglobulinemia or thymoma and hypogammaglobulinemia, this suppression was not observed with purified T-cells of the patients with myeloma were co-cultured with the lymphocytes of normal individuals. In the patients studied, removal of phagocytic mononuclear cells abrogated the suppressor activity and markedly augmented the capacity of the patient's cells to synthesize immunoglobulins in vitro. These observations suggest that one mechanism for the humoral immune deficiency observed in patients with myeloma is a block of polyclonal B-cell terminal maturation by circulating suppressor cells. We have extended these studies into the pathogenesis of the hypogammaglobulinemia observed in myeloma by studying the polyclonal hypogammaglobulinemia associated with Balb/C plasmacytomas, TEPC 183 and SPQC 11. We found that a low molecular weight fraction of extracts of tumor ascites cells derived from mice bearing TEPC 183 and SPQC 11 plasmacytomas suppressed immunoglobulin production by human and mouse cells. In contrast extracts prepared from tumor ascites depleted of mononuclear phagocytes by iron-carbonyl treatment and passage through a magnetic field showed little suppressor activity. In addition, extracts of ascites cells derived from mice bearing plasmacytomas not associated with hypogammaglobulinemia (Modified RPC-5) did not suppress. In addition, extracts of subcutaneous transplanted tumor nodules had no suppressive effect. These results are parallel to those we obtained in human myeloma patients. They are consistent with the idea that the hypogammaglobulinemia of myeloma is due to the formation of immunoregulatory macrophage-like cells which synthesize a suppressor substance.

A broad range of studies directed at the pathogenesis of gluten sensitive enteropathy (GSE) was continued. GSE is a disease of the gastrointestinal tract associated with malabsorption, villous flattening and increased incidence of neoplasia. It is due to the toxic effect of wheat protein (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of patients with GSE carried the HLA-B8 histocompatability antigen. In a similar incidence of this antigen was observed in dermatitis herpetiformis (DH), a skin disease associated with a gastrointestinal lesion similar to that found in GSE. In current studies we have identified a second lymphocyte antigen associated with GSE and DH. This antigen is detected with the use of antisera obtained from mothers of GSE patients and is present on B-cells but not on T-cells or K-cells. The antigen is detected by a procedure that involves the separation of cells to obtain defined B-cell and T-cell fractions using antigen-antibody coated plates or anti-Fab plates and then examination of the purified cells for susceptability to complement-mediated lysis. We have found that virtually all GSE and DH patients are positive for a B-cell antigen whereas none of our 40 control patients are positive. In family studies it was observed that the gene responsible to this antigen does not appear to be linked to the H-complex (HLA-associated genes) since HLA identical siblings are discordant for the antigen. This antigen may be the product of the sought after Ir gene that, from previous studies, is postulated to occur in GSE. In any case, this antigen appears to strongly associate with DH and GSE and can be used to identify the presence of these diseases.

Studies in other laboratories have shown that in addition to HLA-B8 a second HLA linked antigen associated with GSE and DH. This antigen appears to be

the LD antigen DW3 which is detected serologically with define antisera. In current studies we have identified a GSE family with 2 siblings who are MLC identical, i.e., have identical LD antigens. Nevertheless, these siblings are discordant for GSE and the B-cell antigen defined above. The studies conducted with this family indicate that the B-cell antigen detected with maternal antisera are distinct from the LD antigens. In all, the data is compatable with the concept that GSE is due to two separate genes, one associated with or identical to the LD gene and the second not linked to the HLA complex but nevertheless reuslting in a unique B-cell surface protein. Only the presence of both genes can result in disease.

In additional studies on gluten sensitive enteropathy done in conjunction with Dr. Stephen I. Katz, jejunal biopsies from patients with DH were placed in the organ culture in vitro model of GSE previously developed in this laboratory. We found that tissue obtained from DH patients were usually unresponsive to gliadin in vitro (i.e., addition of gliadin did not restrain cell maturation). These studies indicate that the jejunal lesion in DH is quantitatively different than that in GSE. We presume that this is due to differences in the type and number of receptor sites for gliadin on the epithelial cells of patients with the two diseases.

Studies of the cells mediating cytotoxicity reactions continued during this period and led to a much clearer understanding of both mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC) reactions. Human peripheral blood cells were fractionated using ficoll-hypaque gradients, colloidal iron ingestion followed by passage through a magnetic field, passage over Sephadex anti-FAB columns and differential binding to antibody-antigen complex plates. In this way, polymorphonuclear cell-rich populations, purified lymphocytes (macrophage-depleted lymphocytes), purified B-cell populations, T-cell populations containing Fc receptor-positive, SIg-negative cells and T-cell populations depleted of Fc-receptor positive, SIg-negative cells were obtained. All cell populations were found to contain effector cells in MICC using chromated chicken RBC targets, whereas polymorphonuclear cells, macrophages as well as Fc-receptor positive, SIg negative cells but not B-cells or T-cells could function as effector cells for these targets in ADCC.

In contrast to these findings using the chicken red cell target, a far more restrictive effector cell pattern emerged when Chang cells were employed as the target cells. In this case, MICC reactions are mediated exclusively by T-cells. ADCC reactions are mediated exclusively by Fc-receptor positive, SIg negative cells (K-cells). Thus, for the Chang cell target cytotoxic effector cells belong to separate populations. In addition, for this target the B-cell does not mediate ADCC and the K-cell does not mediate MICC. These studies greatly clarify the nature of the effector cells necessary for cytotoxicity for defined targets and open the road to the study of disease states from the point of view of cytotoxic effector function.

Indeed such studies are already well under way and in a completed investigation of cytotoxic effector function in patients with Australia antigen negative chronic hepatitis (CH) and primary biliary cirrhosis (PBC) it was found that

chronic hepatitis patients have normal MICC for Chang targets (T-cell cytotoxicity) and normal spontaneous cytotoxicity for such targets (K-cell cytotoxicity). In contrast, PBC patients had normal MICC and ADCC but reduced spontaneous cytotoxicity (K-cell cytotoxicity). This functional defect involves the loss of a primitive cytotoxic effector function dependent only on cell-cell contact and occurs in spite of the presence of normal numbers of K-cells.

Functional disorders of the K-cell have been revealed by the above studies. K-cells make up 5-10% of the cells in the peripheral blood of normal individuals and are similar to B-cells in that they have Fc receptors. However, they are clearly different from B-cells and other cell types in that they: 1) mediate ADCC for nucleated targets; 2) mediate spontaneous cytotoxicity, 3) are not phagocytic; and 4) have no capacity to form T-rosettes. In addition, studies we have tested purified K-cell populations for the presence of B-cell antigens identified with maternal antisera of inbred families (antigens which are equivalent to murine Ia antigens) and have found that K-cells did not bear B-cell antigens. Thus, these various studies distinguish K-cells from other peripheral cells. The role of the K-cell in a variety of diseases remains to be studied.

Humoral factors playing an important role in immunoregulation have been extensively studied. One of the most striking examples of immunoregulation is found during pregnancy when the histoincompatible fetus and placenta is protected from immunologic attack by the cells and immunoglobulins of the mother. Previously we have shown that human chorionic gonadotropic (HCG), a glycoprotein hormone produced in vast quantities by the syncitiotrophoblast of the placenta, contains potent immunosuppressive activity. This immunosuppression is demonstrable in vitro as a totally reversible inhibition of lymphocyte proliferative responses to nonspecific mitogens like PHA, to specific antigens like Candida, and to allogeneic cells in the mixed lymphocyte reaction. HCG also inhibits the ability of lymphocytes, polymorphonuclear leucocytes and monocytes to kill cellular target cells in a variety of assays. Further studies, however, have shown that highly purified homogenous preparations of HCG do not manifest immunosuppressive activity. Evidence suggests that a small molecular weight compound present in the urine of pregnant women (but not non-pregnant individuals) which co-purified with HCG during its commercial preparation is responsible for the immunosuppressive activity. Other investigators have indicated that alpha-fetoprotein, another protein found in very high concentrations during pregnancy also has potent immunosuppressive properties. Our studies of the effect of highly purified alpha-fetoprotein obtained from an alpha-fetoprotein producing hepatoma have failed to confirm the potent immunosuppressive activity of this material. It is now becoming clear that compounds with the properties of an almost ideal immunosuppressant (non-toxicity, reversibility activity at low concentration) are present during pregnancy but that these materials have not yet been convincingly identified. The identification and purification of this material will prove extremely useful for understanding mechanisms of immunoregulation and may potentially provide a potent compound for clinical control of immune responses.

Another striking example of immunoregulation is the failure of autoimmune reactivity in most individuals. Extensive studies in experimental animals and man have demonstrated an almost universal capacity for autoreaction which is normally held in check by unknown mechanisms. T-lymphocytes have long been known to react to foreign histocompatability antigens by proliferation in vitro (the mixed lymphocyte reaction - MLR). We have found that normal "T" lymphocytes also respond vigorously in vitro to "B" lymphocytes from the same individual, thus giving an "autologous mixed lymphocyte reaction." Under appropriate tissue culture conditions, this auto-reactive MLR approaches the magnitude of the response observed in the more commonly studied allogeneic MLR. In studies of the factors which normally prevent such auto-reactivity from occurring in vivo, we observed that cortisol in physiologic concentrations (4×10^{-7}M) totally abolished this autologous MLR while having no effect on the "T" cell response to foreign histoincompatability cells in the allogeneic MLR. Even 20-fold higher cortisone concentrations only partially inhibit this allogeneic reaction. Thus, concentrations of cortisol found in normal human plasma have profound immunoregulatory effects selectively inhibiting autoimmune reactivity while not interferring with immune responsiveness to foreign tissue antigens.

Proline Transport, Biosynthesis and Metabolism

In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminated ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Hydroxyproline degradation follows a scheme analogous to that for proline. Hydroxyproline is converted first to 3-OH-PC and then to γ-OH glutamate.

Proline and hydroxyproline are first degraded by distinct enzymes to pyrroline-5-carboxylate (PC) and pyrroline-3-OH-5-carboxylate (3-OH-PC), respectively. Our studies showed that the second oxidative step in both degradative pathways (producing glutamate from PC and γ-OH-glutamate from 3-OH-PC) is catalyzed by a single dehydrogenase. Using radioisotopic labelled substrates and recovering specific products we found that PC and 3-OH-PC each competively inhibit the dehydrogenation of the other. In Lineweaver-Burke analysis of enzyme kinetics we found complete inhibition of 3-OH-PC dehydrogenase by PC and of PC dehydrogenase by 3-OH-PC. These findings suggest that a single dehydrogenase oxidizes both PC and 3-OH-PC. To obtain definitive evidence for the single enzyme hypothesis, we studied cells from patients with Type 2 hyperprolinemia, an autosomal recessive inherited metabolic disorder. The biochemical abnormalities in these patients are due to absent PC dehydrogenase activity. Extracts from cultured fibroblasts from Type 2 hyperprolinemia patients were unable to dehydrogenate 3-OH-PC as well as PC. Absent activities were unaltered by dialysis and mixing of normal and patient extracts showed unaltered activity. Thus, the possibility of inhibitors in the Type 2 hyperprolinemia extracts were ruled out. To verify that these defects were not an artifact of cells in long-term

culture, we assayed both PC and 3-OH-PC activities in extracts of fresh peripheral leukocytes. Studies in two patients and their families showed absent, heterozygote or normal leukocyte levels of both PC dehydrogenase and 3-OH-PC dehydrogenase. Linear regression of leukocyte PC dehydrogenase activity on 3-OH-PC dehydrogenase activity in these families and 9 normals produced a correlation coefficient of 0.89. On the basis of these biochemical and genetic studies we conclude that PC and 3-OH-PC are dehydrogenated by a single enzyme.

Proline oxidase, the enzyme which catalyzes the first step in proline degradation, is glutocorticoid inducible, a finding summarized in last year's report. We now have found that proline oxidase undergoes significant changes with mammalian development. In rat liver, adult levels of proline oxidase are 10-15 X fetal levels. The level remains unchanged with parturition but steadily increases and plateaus by age 6-8 weeks. The difference between fetal and adult levels of proline oxidase activity are due to differences in amount of enzyme. There were no inhibitors in fetal liver. Additionally, the affinity of the enzyme for proline was identical in fetal and adult liver. In contrast to the increase in proline oxidase with maturation, hepatic pyrroline carboxylate reductase, a proline biosynthetic enzyme decreases with increasing age. Fetal levels of PC reductase are three-fold the levels in the adult animal. These enzyme changes suggest that during periods of rapid growth, proline is synthesized at an increased rate and the endogenously synthesized proline is spared for protein synthesis. With decreasing rates of growth, proline can be degraded as a source of metabolic energy or it can be converted to metabolic intermediates needed by the animal.

We considered that different tissues with distinct metabolic functions might show distinctive complements of proline synthetic and degradative enzymes. In particular, cartilage and bone with high proline requirements for collagen formation might metabolize proline differently than central organs, e.g., liver. We found the pyrroline-5-carboxylate reductase, the enzyme catalyzing the committed step in proline synthesis is 7-10 X higher in cartilage than in liver. Proline oxidase, which catalyzes the first degradative step, is very active in liver but undetectable in cartilage. Corroborating these enzyme measurements, cartilage was able to oxidize glutamate but unable to oxidize proline to CO_2. Thus, cartilage preserves proline and has greater capacity for synthesizing proline than other tissues. The high synthetic capacity suggested that endogenously synthesized proline may be a significant source for this amino acid in cartilage. In proline incorporation studies in short-term cultures of rat cartilage, we found that as much as 25% of the total proline incorporated was derived from ornithine. Even with physiologic concentrations of exogenous proline, endogenous synthesis contributes significantly to proline used for protein synthesis.

To quantitate the contribution of endogenously synthesized proline to protein synthesis, we turned to a cultured cell line with mutations in the proline synthetic pathway. Chinese hamster ovary cells (CHO-K1) are auxotrophic for proline. The defect is in the inability to form pyrroline-5-carboxylate from ornithine and glutamate. The mechanism to convert pyrroline-5-carboxylate to proline, on the other hand, is intact. Using these cells we were able to measure the rate of incorporation of proline from precursor pyrroline-5-carboxylate without the uncertainties introduced by endogenous synthesis of pyrroline

carboxylate and dilution of specific radioactivities. We found that with media proline at 0.1 mM, incorporation of endogenously synthesized proline was 50% of total proline incorporated. Even with the medium proline at 0.5 mM, the incorporation of endogenously synthesized proline was 20% of total proline incorporated into protein. These studies show that endogenous synthesis makes a significant contribution to proline incorporated into protein and suggests that abnormal regulation or deficient proline biosynthesis may be a pathophysiologic mechanism in metabolic diseases of bone and connective tissue.

Porphyrin Metabolism

The studies of experimental porphyria and the control mechanisms of heme biosynthesis have been extended. The experiments are directed at the primary mechanism by which certain porphyrinogenic compounds alter the heme pool size and lead to induction of hepatic delta-aminolevulinic acid synthetase (ALA synthetase). Since a diminished heme pool size can result from augmented heme turnover or diminished heme synthesis, experiments have been designed to quantitate the effects of variables on these two parameters. The logic of the various types of "pulse experiments" using isotopic precursor to determine total pathway activity has been worked out, as well as the method of calculating this activity from the data.

It has been shown that the turnover curve of total hepatic heme consists of two exponential components with half lives of 9 and 24 hours in normal rats. Two porphyrinogenic compounds, allylisopropylacetamide and 2-allyloxy-3-methylbenzamide have been shown to markedly shorten the half-life of the first component of the hepatic heme turnover curve. Using one specific experimental design selected from the group of well defined logically possible designs, it has been shown that 2-allyloxy-3-methylbenzamide diminishes heme biosynthesis as well as augmenting its turnover.

A protocol for study of the clinical and biochemical effects of intravenously administered hematin in acute intermittent porphyria have been approved by the FDA. Hematin has been prepared for administration to patients.

The Hoesch test has been examined in a number of patients with acute intermittent porphyria and is shown to be of great value as a quick screening test for this disease. It is quicker and simpler than the previously used screening test.

A new enzymatic method for the diagnosis of acute intermittent porphyria, using lysed red cells, is being developed.

Hemopexin levels in the blood of patients with porphyria cutanea tarda have been shown to be decreased. Preliminary data suggest that the levels increase during therapy of the disease by phlebotomy.

Mechanism of Action of Growth Hormone

An understanding of the biochemical events accompanying normal growth (as mediated by growth hormone, for example) may be a prerequisite for understanding

malignant growth. There is evidence that the anabolic action of growth hormone is mediated by a second protein factor - designated somatomedin. Somatomedin may also be one of the serum factors required for the growth of cells in culture. Work in the laboratory over the past year has continued to be directed toward the purification of a somatomedin like peptide (multiplication stimulating activity or MSA) from the conditioned media of a rat liver cell line that grows without serum.

In collaboration with Matthew Rechler, MIAMDD, ^{125}I-MSA has been shown to bind to the cell surface of various cells in culture (chick embryo fibroblasts, human skin fibroblasts, Buffalo rat liver cells). In the case of chick embryo fibroblasts, ^{125}I-MSA is displaced from the cell membrane by somatomedin A, insulin, and proinsulin. The relative ability of MSA, insulin, proinsulin and somatomedin A to compete for MSA binding corresponds to the relative potency of these peptides in stimulating thymidine incorporation into DNA. Also the concentrations of these peptides required for half maximal stimulation of thymidine incorporation into DNA correspond to concentrations required for half maximal displacement of ^{125}I-MSA from the chick embryo fibroblasts. Thus, these studies present evidence of "growth receptor" on the surface of the chick cells in culture.

We have recently shown that human skin fibroblasts grow equally well in sera from growth hormone deficient patients obtained before and after growth hormone treatment. Thus, the serum requirement for the growth of skin fibroblasts in culture does not show growth hormone dependence, i.e., somatomedins do not appear to contribute to the serum macromolecular requirments for the growth of human skin fibroblasts in culture.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4002(c)-09-MET

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Physiology and Pathophysiology of Plasma Protein Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas A. Waldmann, M.D.	Senior Investigator	MET NCI
OTHER:	Warren Strober, M.D.	Senior Investigator	MET NCI
	R. Michael Blaese, M.D.	Senior Investigator	MET NCI
	K. Robert McIntire, M.D.	Senior Investigator	LID NCI
	Samuel Broder, M.D.	Clinical Associate	MET NCI
	Randall S. Krakauer, M.D.	Clinical Associate	MET NCI
	Robert Kerman, M.D.		

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NCI

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were directed toward determining the sequential steps involved in the differentiation of lymphocyte B-cells to immunoglobulin synthesizing and secreting cells. The study was conducted to determine the normal rates of synthesis, transport and catabolism of the plasma proteins, especially the immunoglobulins to determine the physiological factors controlling these metabolic parameters and to determine the pathophysiology of disorders of plasma protein metabolism in patients with neoplastic disease and in patients with hereditary disorders of immunoglobulin metabolism. Developing techniques for the detection of circulating tumor-related antigens has also been an object of study.

PHS-6040
(12-75)

Project Description:

Objectives: The objectives of the study were: to determine the normal rates of synthesis, transport and catabolism of the plasma proteins, especially the immunoglobulins, to determine the physiological factors controlling these metabolic parameters and to determine the pathophysiology of disorders of plasma protein metabolism in patients with neoplastic disease and in patients with hereditary disorders of immunoglobulin metabolism. In addition, studies were directed toward determining the sequential steps involved in the differentiation of lymphocyte B-cells to immunoglobulin synthesizing and secreting cells. Major efforts were directed toward defining the role of suppressor lymphocyte-T-cells in this maturational process. Finally, studies were directed toward developing techniques for the detection of circulating tumor-related antigens.

Methods Employed: The rates of synthesis, catabolism and distribution of the serum proteins were studied using radioiodinated labelled purified proteins. Double antibody radioimmunoassays were established to detect α-fetoprotein, human chorionic gonadotropin and the immunoglobulins IgG, IgA, IgM, IgD and IgE. As indicated below, new techniques have been developed for the study of the terminal differentiation of B-lymphocytes to immunoglobulin secreting cells for the detection of helper T cells in man and for the detection of excessive numbers or reduced numbers of suppressor T cells.

Major Findings: Sensitive quantitative radioimmunoassays for the oncofetal proteins alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG-β) were established in previous years and were shown to be of value in the diagnosis of certain forms of malignancy in that elevated values were demonstrated in a significant number of patients with cancer, but in virtually no patients with benign disorders. The radioimmunoassays for AFP and hCG were evaluated as adjuncts to the diagnosis of testicular malignancy and in monitoring the effectiveness of therapy of this disorder. Ninety percent of 200 patients with germinal neoplasms of the testis had an elevated value of one or both of the marker proteins. Thirty-three patients with a testicular mass were studied with the markers prior to surgery. Both markers were negative in the 29 patients who were ultimately shown to have no germinal tumor of the testis. In contrast, 5 of the 8 patients with a germinal tumor had an elevated serum level of alpha-fetoprotein or hCG. Thus, the use of markers appears to be of value as an adjunct to the diagnosis of cancer of the testis. The radioimmunoassays for these markers were of even greater value in monitoring the effectiveness of therapy of the testicular tumor. The effect of therapy on the clinical course and on the serum levels of AFP and hCG was evaluated in 33 patients who had elevations of both markers prior to therapy. In 9% of the cases there was no clinical response to chemotherapy and the levels of both markers remained stable or increased. In 15% of the cases the marker levels dropped to and remained at the normal level. These patients have had a complete disappearance of their disease with no recurrence. In 30% of the cases the markers declined with moderately effective therapy but both markers remained above the normal range and increased when the tumor recurred clinically. In 33% of the cases, both markers declined but one of the markers

remained above the normal range and the remaining marker fell to normal levels. With recurrence of the tumor one of the markers remained at the normal level whereas the other returned to a very high level. In one patient representing 3% of the series, the tumor markers fell following chemotherapy but the tumor mass failed to diminish. At surgery this patient was shown to have a mature teratoma following therapy with loss of the malignant elements. In 33% of the cases evaluated there was an elevation of one or both of the markers at times following therapy when there was no clinical evidence of residual disease. In each of these cases there was a recurrence of clinically evident tumor within the subsequent year. Thus, it is apparent that all elevated values of AFP or hCG in patients with germ cell tumors reflect residual tumor. With this in mind, it is our view that these markers should be used in protocol studies in which patients with testicular tumors are treated. The presence of a positive marker should be taken into consideration in determining the future course of the patient's therapy. The apparent discordant behavior of the tumor markers in 33% of the patients in which one but not both markers fell to normal levels following therapy and remained normal following recurrence of the tumor suggested that the cells of origin of these markers were probably different. This conclusion, was confirmed in studies performed in conjunction with Dr. Robert Kerman. In these studies an indirect immunoperoxidase test using monospecific rabbit anti-alpha-fetoprotein and monospecific rabbit anti-human chorionic gonadotropin were used to define the cell of origin of these markers. AFP was demonstrated inmononuclear "embryonal" cells within both embryonal carcinoma and entodermal sinus tumor components. hCG in contrast was identified within syncytiotrophoblastic cells in both embryonal carcinoma and choriocarcinoma elements. Thus, these markers had different cell types of origin. In all cases where serum levels of both markers were measured at the time of tissue acquisition there was a complete correlation between the markers present in the tissue sections and the markers elevated in the serum.

A significant effort of the Metabolism Branch over the past years has been directed toward defining the nature of the events of cellular differentiation, cellular interaction and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens and to define the pathological defects in patients with a primary immunodeficiency state or with a malignancy of the T- or B-lymphocyte system. The first of these studies was directed at defining the factors controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo a sequential maturation process from stem cells originating in the marrow to B-lymphocytes. Then following interaction with antigen or mitogen the B-cells mature into immunoglobulin synthesizing plasma cells. The majority of patients with primary immunodeficiency diseases as well as patients with chronic lymphocytic leukemia appear to have a defect in the terminal differentiation of B-lymphocytes into immunoglobulin synthesizing and secreting cells. Previously we developed techniques for the study of the terminal differentiation of such B-lymphocytes. More recently we have focused on the role of regulatory T-cells in the control of this maturational event. In these studies of regulatory T-cells we have used a technique we have developed to define excessive numbers of suppressor T-cells as well as two

new techniques we have developed this year, one to detect and quantitate helper T-cells in man and the second to define deficiency in negative regulatory T-cells. When these techniques were applied to the study of patients with common variable hypogammaglobulinemia the patients were shown to be heterogenous with a variety of different immunological defects. Some of the patients had a circulating inhibitor of B-cell maturation; a significant number of patients had intrinsic defects of the B-cells and a significant subset of patients with common variable hypogammaglobulinemia had an abnormality of regulatory, suppressor T-cells which act to inhibit B-cell maturation and antibody synthesis. This latter subset of patients did not appear to have an intrinsic defect in the maturation of their B-lymphocytes but rather had an abnormal number or abnormally activated set of suppressor T-cells. Patients with thymoma and associated hypogammaglobulinemia were also shown to have excessive numbers of activated suppressor T-cells which may play a role in the hypogammaglobulinemia observed in these patients.

The nature of the defect in patients with isolated IgA deficiency was then investigated using the in vitro technique to study pokeweed mitogen driven terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. The cultured lymphocytes from each of the 14 patients with isolated IgA deficiency studied synthesized normal quantities of IgG and IgM but secreted no IgA into the media. However, 11 of the 14 patients studied synthesized IgA by the 7th day in pokeweed mitogen stimulated cultures as assessed by staining for cytoplasmic IgA using fluorescine labeled anti-IgA antisera. These individuals had normal numbers of IgA specific helper cells and did not have any circulating suppressor cells. Thus, these patients with isolated IgA deficiency can synthesize IgA but appear to have a defect in IgA secretion. A second group of patients involving 3 of the 14 patients did not have lymphocytes with IgA demonstrable in their cytoplasm following culture. When the lymphocytes from these 3 patients were co-cultured with normal lymphocytes and pokeweed mitogen the synthesis of IgA by the normal cells was depressed by 80-100%. Synthesis of IgG and IgM was not depressed. Thus, this smaller group of patients with isolated IgA deficiency did not synthesize IgA and had IgA specific suppressor cells that prevent B-cells from maturing into IgA synthesizing and secreting cells.

As discussed above excessive numbers of suppressor T-cells may be associated with hypogammaglobulinemia. However, it should be noted that such cells normally play a key role in the negative control of immune system in general and in the development of specific immunological tolerance. We have recently developed evidence from an animal model system of autoimmunity that suggests that loss of precursors of suppressor T-cells can lead to autoimmune diseases. In these studies we have used NZB/W mice that have a genetically determined autoimmune disorder similar to that observed in patients with systemic lupus erythematosis. Abnormalities in these mice include defects in the induction and maintenance of tolerance to foreign antigens, the development of antibodies with specificity for nucleic acids, erythrocytes and lymphoid cells, the development of immune complex glomerulonephritis, a Coomb's positive hemolytic anemia and a high incidence of reticuloendothelial neoplasms. To approach the question of T-cell suppressor function in NZB/W mice more directly

we have taken advantage of the observation that splenic T-cells can be activated by Concanavalin A (Con A) to become cells which suppress the immunological functions of other cells. Spleen cells from normal mice as well as from young and adult NZB/W mice cultured in the presence of pokeweed mitogen synthesized 860 $\pm$ 120 ng IgM/10^6 cells/7 days. Concanavalin A (Con A 2 µg/cc) added to the culture caused a 94% suppression of IgM synthesis in adult normal mice and in young NZB/W mice but did not suppress IgM synthesis in adult NZB/W mice. To analyze this defect we studied the suppressor activity of Con A pulsed cells. Spleen cells from normal mice cultured with Con A for 24 hours washed free of Con A produced an 88% suppression of IgM synthesis when co-cultured with normal spleen cells in the presence of pokeweed mitogen. Supernatants of normal spleen cells pulsed with Con A for 24 hours contain a soluble immune response suppressor (SIRS). Spleen cells from both young and adult NZB/W mice could respond to suppressor signals in that they were suppressed by Con A pulsed normal spleen cells or supernates therefrom. When NZB/W mice were studied in the presence of suppressor cells we found that young NZB/W Con A pulsed spleen cells caused suppression of IgM synthesis by spleen cells from a variety of sources whereas adult NZB/W Con A pulsed cells lacked such suppressor activity. We conclude that NZB/W mice lose suppressor T-cell activity as they age and suggest this loss of suppressor cells may be a significant pathogenetic mechanism in the development of autoimmunity in these animals. The demonstration in this study that a soluble immune response to suppressor (SIRS) derived from T-cells following Con A stimulation exists and the observation that NZB/W mice are responsive to this negative signal raised the possibility of treating these mice with SIRS. Indeed, in preliminary studies, the administration of SIRS to the NZB/W mice from an early age led to the development of hypogammaglobulinemia in these animals as well as a virtual prevention of the development of anti-DNA antibodies, Coomb's positive hemolytic anemia, and immune complex nephritis. The occurrence of an abnormality in suppressor T-cell function in NZB/W mice suggest that a similar phenomenon may be characteristic of human systemic lupus erythematosis and other human conditions associated with autoimmunity and further suggests the possibility of treating humans with these disorders in a more physiological manner with the soluble immune response suppressor protein.

The tests of *in vitro* immunoglobulin synthesis and helper and suppressor function were applied to the lymphocytes of patients with a malignancy of the T- or B-cell system. The malignant cells of patients with chronic lymphocytic leukemia are B-lymphocytes with IgM and IgD immunoglobulins on their surface. The chronic lymphocytic leukemia cells could not perform helper T-cell functions and did not act as suppressors in co-culture with normal cells. When cultured *in vitro* these lymphocytes had reduced synthesis of all immunoglobulin classes. However, they preferentially produced significant quantities of IgM when cultured in the presence of pokeweed mitogen. Thus, these cells appear blocked at a stage in development before the production of IgA and IgG molecules. However, the IgM bearing leukemic lymphocytes in these patients were not completely blocked in the sense that they retained the capacity to differentiate after pharmacological stimulation into IgM synthesizing and secreting cells. The peripheral blood leukemic cells in patients with the Sezary syndrome have a T-cell derivation. The peripheral blood T-cells of normal individuals

represent a variety of different types of T-cells that cannot be separated from one another. The monoclonal production of T-cells of a single type in the Sezary syndrome, thus, provides the great opportunity of defining the function of an isolated class of T-cells. The neoplastic lymphocytes from patients with a Sezary syndrome did not synthesize detectable immunoglobulins, in addition, they did not have suppressor cell capacity. However, the lymphocytes from 3 of the 4 patients with the Sezary syndrome studied were shown to have a marked capacity to function as helper T-cells. They appeared to represent a monoclonal expansion of the helper T-cell population.

Patients with multiple myeloma have been shown to have a significant reduction in the serum levels of polyclonal immunoglobulins and in the capacity to synthesize antibody in response to antigenic challenge. When the in vitro immunoglobulin biosynthesis test was applied to the peripheral blood lymphocytes from patients with myeloma they showed profound depression of immunoglobulin synthesis to approximately 10% of the normal mean values. Peripheral blood lymphocytes from the majority of patients with myeloma tested suppressed the polyclonal immunoglobulin synthesis of normal lymphocytes when co-cultured with them. However, in contrast to the observations in patients with common variable hypogammaglobulinemia or thymoma and hypogammaglobulinemia, this suppression was not observed with purified T-cells of the patients with myeloma were co-cultures with the lymphocytes of normal individuals. In the patients studied, removal of phagocytic mononuclear cells abrogated the suppressor activity and markedly augmented the capacity of the patients' cells to synthesize immunoglobulins in vitro. These observations suggest that one mechanism for the humoral immune deficiency observed in patients with myeloma is a block of polyclonal B-cell terminal maturation by circulating suppressor cells. We have extended these studies into the pathogenesis of the hypogammaglobulinemia observed in myeloma by studying the polyclonal hypogammaglobulinemia associated with Balb/C plasmacytomas, TEPC 183 and SPQC 11. We found that a low molecular weight fraction of extracts of tumor ascites cells derived from mice bearing TEPC 183 and SPQC 11 plasmacytomas suppressed immunoglobulin production by human and mouse cells. In contrast extracts prepared from tumor ascites depleted of mononuclear phagocytes by iron-carbonyl treatment and passage through a magnetic field showed little suppressor activity. In addition, extracts of ascites cells derived from mice bearing plasmacytomas not associated with hypogammaglobulinemia (modified RPC-5) did not suppress. In addition, extracts of subcutaneous transplanted tumor nodules had no suppressive effect. These results are parallel to those we obtained in human myeloma patients. They are consistent with the idea that the hypogammaglobulinemia of myeloma is due to the formation of immunoregulatory macrophage-like cells which synthesize a suppressor substance.

Significance to Biomedical Research: The techniques developed for the study of the terminal differentiation of B-cells and for helper and suppressor T cells is of the same value in the study of B-cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus-dependent lymphocyte (T-cell) maturation. Thus, in vitro techniques are now available to evaluate both arms of specific immune response in man. One can now study the physiological factors regulating this response, one can define defects in the

process in patients with immunodeficiency disorders or neoplasia of the B-cell or T-cell lymphocyte system. In addition, such studies can be used to determine the effect of chemotherapeutic or other agents of the terminal differentiation of B-cells in parallel with studies of the effects of these agents on T-cells so that one can define agents which preferentially affect T-cell immunity and those which preferentially effect B-cell immunity. Detection of circulating tumor-related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of the neoplastic disease and determining the efficacy of therapy of these disorders.

Proposed Course: Studies directed toward understanding the pathways and controlling mechanisms of the synthesis, transport and catabolism of serum proteins will be continued. Special emphasis will be placed on defining the physiological and pathophysiological factors controlling the sequential development of B cells into immunoglobulin synthesizing cells. Special emphasis will also be placed on the development of new techniques for the detection of circulating tumor-related antigens. These latter studies will include the evaluation of the synthetic rates of catabolism of such tumor-related antigens.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4003(c)-08-MET

PERIOD COVERED
July 1, 1975 through June 20, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald P. Tschduy, M.D.	Senior Investigator	MET	NCI
OTHER:	Joel M. Lamon, M.D.	Clinical Associate	MET	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives are to study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-host relationships as well as primary diseases of porphyrin metabolism.

PHS-6040
(12-75)

Project Description:

Objectives: To study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-host relationships as well as primary diseases of porphyrin metabolism.

Methods Employed: Quantitative enzyme determinations, chemical determination of porphyrins and porphyrin precursors, isolation, purification and measurement of tissue heme, use of tissue preparations and isotope methods in studying metabolic pathways and computer and mathematical analysis of certain mathematical models.

Major Findings: The studies of experimental porphyria and the control of heme biosynthesis have been extended. The experiments are directed at the primary mechanism by which certain porphyrinogenic compounds alter the heme pool size and lead to induction of hepatic delta-aminolevulinic acid synthetase (ALA synthetase). Since a diminished heme pool size can result from augmented heme turnover or diminished heme synthesis, experiments have been designed to quantitate the effects of variables on these two parameters. The logic of the various types of "pulse experiments" using isotopic precursor to determine total pathway activity has been worked out, as well as the method of calculating this activity from the data.

It has been shown that the turnover curve of total hepatic heme consists of two exponential components with half lives of 9 and 24 hours in normal rats. Two porphyrinogenic compounds, allylisopropylacetamide and 2-allyloxy-3-methylbenzamide have been shown to markedly shorten the half-life of the first component of the hepatic heme turnover curve. Using one specific experimental design selected from the group of well defined logically possible designs, it has been shown that 2-allyloxy-3-methyl-benzamide diminishes heme biosynthesis as well as augmenting its turnover.

A protocol for study of the clinical and biochemical effects of intravenously administered hematin in acute intermittent porphyria has been approved by the FDA. Hematin has been prepared for administration to patients.

The Hoesch test has been examined in a number of patients with acute intermittent porphyria and is shown to be of great value as a quick screening test for this disease. It is quicker and simpler than the previously used screening test.

A new enzymatic method for the diagnosis of acute intermittent porphyria, using lysed red cells, is being developed.

Hemopexin levels in the blood of patients with porphyria cutanea tarda have been shown to be decreased. Preliminary data suggest that the levels increase during therapy of the disease by phlebotomy.

Significance to Biomedical Research: Mechanisms of the control of metabolic pathways and the synthesis of enzyme in normal and diseased tissues are being elucidated. The findings have been directed at both theoretical and practical goals. In the former realm, models are being developed to explain the behavior of the pathway and its control system under normal and pathological conditions. In the practical realm, methods of diagnosis and treatment of the porphyrias are being evolved. The abnormalities of the control of heme biosynthesis in tumors and host livers which have previously been demonstrated in this laboratory will be further studied by the methods developed for experimental and human porphyria.

Proposed Course: (A) To continue studies of the factors controlling heme and heme-enzyme synthesis in normal and pathologic tissues. (B) To continue studies of biochemical abnormalities of porphyria in man and experimental animals. (C) To study control mechanisms of heme synthesis in tumors and livers of tumor-bearing hosts.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4004(c)-07-MET

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Amino Acid Biosynthesis and Degradation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James M. Phang, M.D.	Senior Investigator	MET	NCI
OTHER:	S. Peter Nissley, M.D.	Senior Investigator	MET	NCI
	Edward Kowaloff, M.D.	Clinical Associate	MET	NCI
	Robert J. Smith, M.D.	Clinical Associate	MET	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	3	4

SUMMARY OF WORK (200 words or less - underline keywords)

An important factor in cellular growth and metabolism is the biosynthesis, degradation, and transport of amino acids. This project emphasizes the regulatory interplay in amino acid metabolism.

PHS-6040
(12-75)

Project Description:

Objectives: The biosynthesis, degradation and transport of amino acids play an important role in cellular growth and metabolism. The mechanisms by which these processes are regulated in normal and malignant cells are poorly understood. This project emphasizes the regulatory interplay in amino acid metabolism. It is relevant to Objective 3, Approaches 1 and 5 of the National Cancer Program.

Methods Employed: We are primarily using animal cells maintained in long-term tissue culture. Cells can be cloned to insure a genetically homogeneous population. Mutants are isolated by standard techniques. A variety of biochemical techniques have been utilized to study amino acid biosynthesis and degradation in cultured cells. Uptake of amino acids may be assessed by using labeled L-amino acids and rates of protein and collagen synthesis can be obtained by incubating cells with L-proline-C^{14} and separation of the radioisotopically incorporated amino acids as proline and hydroxyproline by thin layer chromatography. The enzymes for biosynthesis and degradation of proline are assayed by using appropriate labeled precursors and isolating the products by ion exchange chromatography. Our methods are sensitive enough to measure these enzymes in leucocytes isolated from 10 ml of blood.

Major Findings: In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Hydroxyproline degradation follows a scheme analogous to that for proline. Hydroxyproline is converted first to 3-OH PC and then to γ-OH glutamate.

Proline and hydroxyproline degradation: A single dehydrogenase enzyme- Proline and hydroxyproline are first degraded by distinct enzymes to pyrroline-5-carboxylate (PC) and pyrroline-3-OH-5-carboxylate (3-OH-PC) respectively. Our studies showed that the second oxidative step in both degradative pathways (producing glutamate from PC and γ-OH-glutamate from 3-OH-PC) is catalyzed by a single dehydrogenase. Using radioisotopic labelled substrates and recovering specific products we found that PC and 3-OH-PC each competively inhibit the dehydrogenation of the other. On Lineweaver-Burke analysis of enzyme kinetics we found competitive inhibition of 3-OH-PC dehydrogenase by PC and of PC dehydrogenase by 3-OH-PC. These findings suggest that a single dehydrogenase oxidizes both PC and 3-OH-PC. To obtain definitive evidence for the single enzyme hypothesis, we studied cells from patients with Type 2 hyperprolinemia, an autosomal recessive inherited metabolic disorder. The biochemical abnormalities in these patients are due to absent PC dehydrogenase activity. Extracts from cultured fibroblasts from Type 2 hyperprolinemia patients were unable to dehydrogenate 3-OH-PC as well as PC. Absent activities were unaltered by dialysis and mixing of normal and patient extracts showed

unaltered activity. Thus the possibility of inhibitors in the Type 2 hyperprolinemia extracts were ruled out. To verify that these defects were not an artifact of cells in long-term culture, we assayed both PC and 3-OH-PC activities in extracts of fresh peripheral leukocytes. Studies in two patients and their families showed absent, heterozygote or normal leukocyte levels of both PC dehydrogenase and 3-OH-PC dehydrogenase. Linear regression of leukocyte PC dehydrogenase activity on 3-OH-PC dehydrogenase activity in these families and 9 normals produced a correlation coefficient of 0.89. On the basis of these biochemical and genetic studies we conclude that PC and 3-OH-PC are dehydrogenated by a single enzyme.

Ontogenic changes in the enzymes of proline metabolism - Proline oxidase, the enzyme which catalyzes the first step in proline degradation, is glucocorticoid inducible, a finding summarized in last year's report. We now have found that proline oxidase undergoes significant changes with mammalian development. In rat liver, adult levels of proline oxidase are 10-15 X fetal levels. The level remains unchanged with parturition but steadily increases and plateaus by age 6-8 seeks. The difference between fetal and adult levels of proline oxidase activity are due to differences in amount of enzyme. There were no inhibitors of fetal activity. Additionally, the affinity of the enayme for proline was identical in fetal and adult liver. In contrast to the increase in proline oxidase with maturation, hepatic pyrroline carboxylate reductase, a proline biosynthetic enzyme decreases with increasing age. Fetal levels of PC reductase are three-fold the levels in the adult animal. These enzyme changes suggest that during periods of rapid growth, proline is biosynthesized and spared for protein synthesis. With decreasing rates of growth, proline can be degraded as a source of metabolic energy or it can be converted to metabolic intermediates needed by the animal.

Tissue differences in proline metabolism - We considered that different tissues with distinct metabolic function might show distinctive complements of proline synthetic and degradative enzymes. In particular, cartilage and bone, with high proline requirements for collagen formation might metabolize proline differently than central organs, e.g., liver. We found the pyrroline-5-carboxylate reductase, the enzyme catalyzing the committed step in proline synthesis is 7-10 X higher in cartilage than in liver. Proline oxidase, which catalyzes the first degradative step in very active in liver but undetectable in cartilage. Corroborating these enzyme measurements, cartilage was able to oxidize glutamate but unable to oxidize proline to CO_2. Thus, cartilage preserves proline and has greater capacity for synthesizing proline than other tissues. The high synthetic capacity suggested that endogenously synthesized proline may be a significant source for this amino acid in cartilage. In protein incorporation studies in short-term cultures of rat cartilage, we found that as much as 25% of the total proline incorporated was derived from ornithine. Even with physiologic concentrations of exogenous proline, endogenous synthesis contributes significantly to proline used for protein synthesis.

Quantitation of exogenous and endogenously synthesized proline in protein synthesis - To quantitate the contribution of endogenously synthesized proline

to protein synthesis, we turned to a cultured cell line with mutations in the proline synthetic pathway. Chinese hamster ovary cells (CHO-K1) are auxotrophic for proline. The defect is in the inability to form pyrroline-5-carboxylate from ornithine and glutamate. The capability to convert pyrroline-5-carboxylate to proline, on the other hand, is intact. Using these cells we measured the rate of incorporation of proline from precursor pyrroline-5-carboxylate without concern that endogenous synthesis of either pyrroline carboxylate or proline would dilute the specific activities. We found that with media proline at 0.1 mM incorporation of endogenously synthesized proline was 50 percent of total proline incorporated. Even with medium proline at 0.5 mM, the use of endogenously synthesized proline was 20 percent of total proline incorporated into protein. These studies show that endogenous synthesis makes a significant contribution to proline used for protein synthesis and suggests that abnormal regulation or deficient proline biosynthesis may be a pathophysiologic mechanism in metabolic diseases of bone or connective tissue.

Significance to Biomedical Research: The regulation of amino acids for protein synthesis may play an important role in controlling cell growth. The enzymes involved in amino acid biosynthesis and degradation are affected by genetic, nutritional and hormonal factors. Our studies on the proline metabolic enzymes are contributing to the understanding of these factors. Specifically, genetic defects in the proline metabolic enzymes have elucidated some of the physiologic derangements secondary to a metabolic block. Elucidation of the molecular mechanisms for regulating proline biosynthesis and degradation will lead to an understanding of biochemical regulation in mammalian cells.

Proposed Course: Our findings that proline oxidase 1) undergoes ontogenic changes, 2) is glucocorticoid inducible and 3) has activity levels different in various tissues all suggest that proline degradation may serve a vital metabolic function. We are emphasizing the role of proline in gluconeogenesis. A method has been developed for quantitating labeled glucose from a number of precursor ^{14}C-labeled amino acids. Whether proline directly or indirectly contributes to the formation of glucose is being currently investigated. A number of cultured cell lines in which the proline degradative enzymes have been defined will be of special interest. LLC-K1 cells (proline oxidase$^+$, PC dehydrogenase$^-$ and Type 2 hyperprolinemia fibroblasts (proline oxidase$^-$, PC dehydrogenase$^+$) will be compared to normal human fibroblasts (proline oxidase$^-$, PC dehydrogenase$^+$). The finding that the second degradative step for both proline and hydroxyproline shares a common dehydrogenase suggests that intracellular concentration of proline will regulate the degradation of hydroxyproline and vice versa. The end product of proline degradation is alpha-ketoglutarate whereas hydroxyproline is degraded to pyruvate and glyoxylate since the metabolic functions of these end products are distinct, the regulation of proline and hydroxyproline degradation may affect a number of physiologic and pathophysiologic functions.

Tissue differences in levels of PC reductase, the enzyme catalyzing the committed step in proline synthesis prompts us to further define the role of

endogenous synthesis in proline incorporation into collagen and other proteins. The effect of a number of anabolic hormones (somatomedins, growth hormone, insulin) will be studied in an attempt to correlate levels of PC reductase and rates of collagen synthesis. The cultured human peripheral lymphocyte may also be of interest in this regard. We previously showed that PC reductase in phytohemagglutinin-stimulated lymphocytes increases 15-20 fold in activity. We plan to use this phenomenon as a measure of maximal inducibility of the enzyme and will screen a variety of metabolic defects to ascertain whether inducibility may serve as a discriminant function in disease.

We are studying cultured osteogenic sarcoma cells (the gift of Dr. Steven Rosenberg, Surgery Branch, NCI) to define abnormalities of either proline metabolism or collagen synthesis in tumor cells. These cells are of special interest since the parent cartilage cells metabolize proline distinctly different than cells from liver or kidney.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4014(c)-07-MET

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of the Immune Response in Normal and Pathological States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Warren Strober, M.D.	Senior Investigator	MET	NCI
OTHER:	David L. Nelson, M.D.	Senior Investigator	MET	NCI
	Neil M. Ellison, M.D.	Clinical Associate	MET	NCI
	Stephen I. Katz, M.D.	Senior Investigator	MET	NCI
	Thomas A. Waldmann, M.D.	Senior Investigator	MET	NCI

COOPERATING UNITS (if any)

Division of Immunology, National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Metabolism Branch

SECTION

Immunophysiology Section

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were directed toward the analysis of immune response in normal humans and patients with a variety of immune disorders, as well as analysis of humoral and cellular immune responses of experimental animals.

PHS-6040
(10-75)

Project Description:

Objectives: Studies were directed toward the analysis of humoral and cellular immune responses of experimental animals with particular emphasis on the study of experimental models of human disease. Studies were directed toward the analysis of immune response in normal humans and patients with a variety of immune disorders. This included the study of patients with gluten-sensitive enteropathy, dermatitis herpetiformis, inflammatory bowel disease, as well as patients with a variety of immune deficiency diseases, such as ataxia telangiectasia, common variable hypogammaglobulinemia and intestinal lymphangiectasia. These diseases are each associated with a high incidence of reticuloendothelial malignancy and the mechanism underlying this association was sought.

Methods Employed: A host of techniques were used to assess immunologic function of experimental animals and in patients with disorders of the immune system. Circulating B-cells in peripheral blood and tissue were enumerated with carefully prepared fluorescein-conjugated antiserums with Ig specificity. Circulating T-cells were enumerated by sheep red blood cell rosette techniques. Antibody responses were assessed with microhemagglutinination techniques and in vitro proliferative responses were assessed with tritiated thymidine incorporation following exposure to specific and nonspecific mitogens. The presence of mitogen induced cytotoxic effector cells were assessed with a ^{51}chromium release assays, using red cell and nucleated, metabolically active cell targets. The presence of antibody-mediated cytotoxic effector cells was assessed with ^{51}chromium release assays using red cell and nucleated cell targets in the presence of appropriate antibodies. Cells were isolated with the use of Sephadex anti-Fab columns, rosetting techniques, use of colloidal iron followed by passage through a magnetic field and differential binding to antigen-antibody plates. Short term (1-2 hour) organ culture was used in conjunction with a solid phase immunoabsorption technique to measure local mucosal immunoglobulin synthesis. Long term (24-48 hour) organ culture was used to study in vitro models of intestinal disease. Radioimmunoassays in conjunction with lymphocyte culture in the presence of mitogen was used to measure terminal B-cell differentiation. Patients with immunologic disorders were studied with immunoelectrophoresis, radial immunodiffusion and metabolic turnover techniques for measurement of immunoglobulin synthesis and catabolism.

Major Findings: Gluten-sensitive enteropathy - A broad range of studies directed at the pathogenesis of gluten sensitive enteropathy (GSE) were continued. GSE is a disease of the GI tract associated with malabsorption, villous flattening and increased incidence of neoplasia. It is due to the toxic effect of wheat protein (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of patients with GSE carried the HLA-B8 histocompatability antigen. A similar incidence of this antigen was observed in dermatitis herpetiformis, a skin disease associated with a gastrointestinal lesion similar to that found in GSE. In current studies we have identified a second lymphocyte antigen associated with GSE and DH. This antigen is detected with the use of antisera obtained from

mothers of GSE patients and is present on B cells but not on T-cells or K-cells. The antigen is detected by a procedure that involves the separation of cells to obtain defined B-cell and T-cell fractions using antigen-antibody coated plates or anti-Fab plates and then examination of the purified cells for susceptability to complement-mediated lysis. We have found that virtually all GSE and DH patients are positive for a B-cell antigen whereas none of our 40 control patients are positive. In family studies it was observed that the gene responsible for this antigen does not appear to be linked to the H-complex (HLA-associated genes) since HLA identical sibs are discordant for the antigen. This antigen may be the product of the sought after Ir gene that, from previous studies, is postulated to occur in GSE. In any case, this antigen appears to be strongly associated with DH and GSE and can be used to identify the presence of these diseases.

Studies in other laboratories have shown that in addition to HLA-B8, a second HLA-linked antigen associated with GSE and DH. This antigen appears to be the LD antigen, DW3, which is detected serologically with defined antisera. In current studies we have identified a GSE family with 2 siblings who are MLC identical, i.e., have identical LD antigens. Nevertheless these siblings are discordant for GSE and the B-cell antigen defined above. The studies conducted with this family indicate that the B-cell antigen detected with maternal antisera are distinct from the LD antigens.In addition, the data is compatable with the concept that GSE is due to two separate genes, one associated with or identical to the LD gene and the second not linked to the HLA complex but nevertheless resulting in a unique B-cell surface protein. Only the presence of both genes can result in disease.

In addition studies on gluten-sensitive enteropathy done in conjunction with Dr. Stephen I. Katz of the Dermatology Branch, jejunal biopsies from patients with DH were studied in organ culture to determine their behavior in the in vitro model of GSE previously developed in this laboratory. We found that tissue obtained from DH patients were usually unresponsive to gliadin in vitro (i.e., addition of gliadin did not restrain epithelial cell maturation). These studies indicate that the jejuanl lesion in DH is quantitatively different than that in GSE. We presume that this is due to differences in the type and number of receptor sites for gliadin on the epithelial cells of patients with the two diseases.

Secretory component deficiency - The study of patients with secretory component deficiency was continued. As reported previously a patient has been identified with absent secretory IgA, associated with normal levels of serum IgA. This patient has abnormal synthesis of IgA in the jejunal mucosa but has circulating lymphoid cells which can synthesize IgA in vitro. In contrast to patients with selective IgA deficiency this patient has no secretory component in saliva or jejunal secretions. In current studies, the family of the patient mentioned above has been studied and a cousin identified who has a similar lesion. It is of interest that the cousin differs from the original patient by the fact that he has no chronic GI disease or candidiasis but instead suffers from mild chronic respiratory symptoms. The original patient had lymphocytes with defective MIF production response to

exposure to candida antigen whereas the cousin had normal MIF production after such exposure. Presence or absence of associated immunologic defects such as MIF production capacity appear to determine whether or not patients with secretory component deficiency have clinical disease. In any case these patients with secretory component deficiency constitute an important "experiment of nature" in that they indicate that homing and the differentiation of IgA cells to the gut depends on the synthesis of secretory component.

Agammaglobulinemia and autoimmunity - A new subclass of common variable hypogammaglobulinemia has been identified. These patients have agammaglobulinemia associated with autoimmune manifestations such as hemolytic anemia, pulmonary fibrosis, and CNS vasculitis. Unlike other patients with common variable hypogammaglobulinemia, they lack circulating B-cells (surfacing immunoglobulin-bearing cells) while they retain Fc-positive lymphocytes which are functionally capable of mediating ADCC reactions against nucleated, metabolically active target cells. In addition, these patients unlike most patients with common variable hypogammaglobulinemia, do not demonstrate excessive suppressor T-cell function in that co-culture of their cells with B-cells obtained from normals does not inhibit normal B-cell immunoglobulin synthesis. These patients appear to have unique defects in lymphoid cell maturation that preceeds B-cell development but does not affect K-cell development. The lack of suppressor T-cell function and autoimmunity allows one to suggest that the autoimmunity seen in these patients is related to a defect in suppressor T-cell function.

Studies on the mechanism of cytotoxicity reactions - Studies of the cells mediating cytotoxicity reactions continued during this period and led to a clearer understanding of both mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC) reactions.

Human peripheral blood cells were fractionated using ficoll-hypaque gradients, colloidal iron ingestion followed by passage through a magnetic field, passage over Sephadex anti-Fab columns and differential binding to antibody-antigen complex plates. In this way, polymorphonuclear cell-rich populations, purified lymphocytes (macrophage-depleted lymphocytes), purified B-cell populations, T-cell populations containing Fc receptor-positive, sIg-negative cells and T-cell populations depleted of Fc-receptor positive, sIg-negative cells were obtained. All cell populations were found to contain effector cells in MICC using chromated chicken RBC targets, whereas polymorphonuclear cells, macrophages as well as Fc-receptor positive, sIg-negative cells, but not B-cells or T-cells could function as effector cells for these targets in ADCC.

In contrast to these findings using the chicken red cell target, a far more restricted effector cell pattern emerged when Chang cells were employed as the target cells. In this case, MICC reactions are mediated exclusively by T-cells and ADCC reactions are mediated exclusively by Fc-receptor positive, sIg negative cells (K-cells). Thus, for the Chang cell target cytotoxic effector cells belong to separate populations; in addition, for this target the B-cell does not mediate ADCC and the K-cell does not

mediate MICC. These studies greatly clarify the nature of the effector cells for cytotoxicity of defined targets and open the road to the study of disease states from the point of view of cytotoxic effector function.

Indeed such studies are already well under way and in a completed investigation of cytotoxic effector function in patients with Australia antigen negative chronic hepatitis (CH) and primary biliary cirrhosis (PBC) it was found that chronic hepatitis patients have normal MICC for Chang targets (T-cell cytotoxicity) and normal spontaneous cytotoxicity for such targets (K-cell cytotoxicity). In contrast, PBC patients had normal MICC and ADCC but reduced spontaneous cytotoxicity (K-cell cytotoxicity). This functional defect involved the loss of a primitive cytotoxic effector function dependent only on cell-cell contact and occurs in spite of the presence of normal numbers of K-cells.

Functional disorders of the K-cell have been revealed by the above studies. K-cells make up 5-10% of the cells in the peripheral blood of normal individuals and are similar to B-cells in that they have Fc receptors. However, they are clearly different from B-cells and other cell types in that they: 1) mediate ADCC for nucleated targets; 2) mediate spontaneous cytotoxicity; 3) are not phagocytic; and 4) have no capacity to form T-rosettes. In additional studies we have tested purified K-cell populations for the presence of B-cell antigens identified with maternal antisera of inbred families (antigens which are equivalent to murine Ia antigens) and have found that K-cells did not bear B-cell antigens. Thus these various studies distinguish K-cells from other peripheral cells. The role of the K-cell in a variety of diseases remains to be studied.

Significance to Biomedical Research: Studies of gluten-sensitivity have provided important new insights in the nature of immune responses. This disease is the only disease in which a high association with histocompatibility-related antigens has been found in which the toxic agent, gluten protein, is known. This disease is, therefore, an important human model for the study of the association of histocompatibility antigens and disease. In the studies conducted during the past year we have identified a lymphocyte surface antigen which may be the product of a sought after immune response (Ir) gene which was earlier postulated to underly the pathogenesis of GSE. This discovery points the way to the proof of the Ir gene hypothesis since the antisera recognizing the GSE-associated antigen can be used in a variety of experimental systems to block the afferent and efferent mechanisms responsible for gluten-toxicity. For instance, in preliminary studies we have shown that maternal antisera recognizing the GSE associated antigen can block the action of gluten in the organ culture model of gluten enteropathy referred to above.

Studies of lymphocyte function, particularly with regard to cytotoxic potential of cells active in MICC and ADCC will lead to an increasingly clear understanding of many diseases. In cytotoxic studies conducted here the K-cells are clearly differentiated from B-cells in regard to ADCC and from T-cells with regard to MICC. In addition, the K-cell is distinct from the

B-cell in that it does not bear the murine equivalent of Ia antigens. In one disease state, primary biliary cirrhosis abnormalities of K-cell function have been identified in the presence of normal B and T-cell function whereas in a second disease state, K-cell function is intact but B-cell functions are absent. These studies point to the independence of the K-cell and the fact that the K-cell is a cell type in mammals with an origin and fate which is distinct from B-cells and T-cells. The role of this cell in immune processes and causation and prevention of disease can now be more fruitfully studied.

It is well known that patients with GSE and inflammatory bowel disease as well as patients with immunodeficiency are subject to a greatly increased risk of neoplasia. A variety of evidence points to the immunologic system as an important bulwark against the development of neoplasia. Studies of the diseases characterized by immunologic abnormalities will provide insight into how the immunologic system acts to prevent neoplasia as well as how immune responses may be manipulated to prevent neoplasia.

Proposed Course: Investigation of the role immunologic factors in gastrointestinal disease will be pursued. Attempts will be made to further study the significance of the newly discovered gluten sensitive enteropathy-associated antigen identifiable with maternal antisera. In particular it will be important to study whether or not this antigen has specificity for the toxid material operative in GSE,namely gluten protein. If this proves to be so, then the proposition that GSE is initiated by an abnormal immune response gene will be greatly enhanced. Studies utilizing the in vitro model of GSE developed previously will be conducted in order to demonstrate that the effector limb of gluten toxicity is dependent on the presence of gluten-specific receptor sites on the surface of gut epithelial cells. In this regard antisera recognizing the GSE-associated antigen will be used to try to block the appropriate receptor sites and thereby prevent toxicity in vitro. Other studies of patients with GSE will be conducted in order to show more clearly that the inheritance of the disease is dependent on an HLA associated gene as well as on the inheritance of the GSE-associated gene not linked to HLA. This study will be facilitated by the use of identifying antisera with specificity for HLA- and GSE-associated genes. Studies of patients with DH will be continued; an attempt to show that receptor sites in the skin with specificity for gluten protein exist and are responsible for the characteristic deposition of IgA in the skin of DH patients; it will be important to show whether or not this receptor site is similar to the receptor site on the surface of lymphocytes identified with the maternal antisera mentioned above.

In the area of inflammatory bowel disease, studies of cytotoxic effector cell potential will be conducted. In this regard, heavy use will be made of the fact that using Chang cell targets the effector cells responsible for cytotoxicity have been very clearly identified. In this way abnormalities in B-cell, T-cell or K-cell function using appropriate targets will be identified in patients.

Fundamental studies of gut immunology will be continued using an in vitro model of B-cell differentiation. In this regard PWM and LPS-driven IgM and IgA production in vitro will be studied using radioimmunoassay techniques developed for the study of human lymphoid cells. Studies of suppressor cell potential of various tissue including T-cells derived from the gastrointestinal tract on IgA production in vitro. Similar studies will be extended to human lymphoid material obtained at surgery and frozen for future use. In this way the suppressor and helper function of gut-associated lymphoid tissue obtained from humans with various diseases will be studied.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-ZO1-CBC-4015(c)-05

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Michael Blaese, M.D.	Senior Investigator	M NCI
OTHER:	Andrew V. Muchmore, M.D.	Senior Investigator	M NCI
	E. Clinton Lawrence, M.D.	Clinical Associate	M NCI
	David G. Poplack, M.D.	Senior Investigator	PO NCI
	David Ilfeld, M.D.	Research Associate	M NCI
	Luiz Barbosa, M.D.	Senior Investigator	ID NINCDS
	David Fucillo, M.D.	Research Associate	ID NINCDS
	Randall S. Krakauer, M.D.	Clinical Associate	M NCI
	David L. Nelson, M.D.	Senior Investigator	M NCI
	Warren Strober, M.D.	Senior Investigator	M NCI
	Thomas A. Waldmann, M.D.	Senior Investigator	M NCI

COOPERATING UNITS (if any)

Pediatric Oncology Branch, DCT, NCI, and Infectious Disease Branch, NINCDS.

LAB/BRANCH
Metabolism Branch

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5½	3	2½

SUMMARY OF WORK (200 words or less - underline keywords)

This study is directed toward determining mechanisms important to the development and function of humoral and cellular immune responses; the nature of deficiency in immune function; and developing approaches to prophylaxis and/or therapy of infections and neoplastic disease associated with immune processes.

PHS-6040

Project Description:

Objectives: The objectives of this study were to determine mechanisms important to the development and function of both humoral and cellular immune response in animals and man; to determine the nature of the deficiency in immune immune function characterizing such disease states as the Wiskott-Aldrich syndrome, intestinal lymphangiectasia and advanced malignancy; and, to develop approaches to prophylaxis and/or therapy of the infectious and neoplastic disease frequently associated with defective immune processes. This work is directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: Antibody responses of experimental animals and man were determined after immunization with bacterial vaccines such as brucella abortus, S. typhosa, and pertussis; with particulate cellular antigens such as sheep, burro, and chicken RBC's; with purified protein antigens such as KLH, diphtheria and tetanus toxoids, guinea pig-γ-globulin and bovine γ-globulin; with purified polysaccharide antigens such as pneumococcal polysaccharides types I, II, III, and the V_i antigen of E. coli. The antibodies were detected by standard techniques or by newly developed micro methods of bacterial agglutination, red cell hemolysis, and passive hemagglutination using $CrCl_3$ as an agent to couple the antigens to a carrier RBC surface. In addition, modifications of the Farr antigen binding assay were used.

Cellular immune responses were measured by delayed hypersensitivity skin tests, contact sensitivity to DNCB, and skin allografts. Lymphocyte transformation in vitro was carried out utilizing non-specific mitogens such as PHA, PWM and staph filtrate; specific antigens such as SLO, SKSD, diphtheria and tetanus toxoids, KLH and candida albicans; and allogeneic cells in mixed leukocyte culture. Lymphocyte function in vitro was also measured by production of mediators of cellular immunity such as lymphotoxin, MIF, and lymphocyte derived chemotactic factor for monocytes (LDCF) and by cytotoxicity reactions with antibody sensitized target cells or with non-specific mitogen activated effector lymphocytes.

Studies of the characteristics of various cell surface receptors on immunocompetent lymphocytes and monocytes-macrophages utilized auto-radiography, immunofluorescence microscopy, and cellular rosette formation.

Major Findings: Further studies of the immunoregulatory role of T-cells have been pursued in an experimental animal model of humoral immunodeficiency. In avian species, a central lymphoid organ, the Bursa of Fabricius, is the site of initial differentiation of B-lymphocytes. Removal of the Bursa in the new hatched period results in agammaglobulinemia in the birds that survive. This agammaglobulinemic state is characterized by total deficiency of B-lymphocytes and plasma cells, absence of germinal centers in the lymphoid tissue of these birds, and a total absence of IgM, IgA, and IgG. Cellular immune function, however, is preserved. T-cells from agammaglobulinemic chickens were found to have profound effects on humoral immune responses when

transplanted into normal birds. Such T-cell graft recipients also developed agammaglobulinemia despite the continued presence of a Bursa of Fabricius in these birds. This "infectious agammaglobulinemia" was characterized by total absence of IgM, IgA and IgG but differed from that induced by bursectomy by the presistence of B-lymphocytes and germinal centers in the lymphoid tissues. Thus, the immune defect in these T-cell graft recipients closely parallels the defect observed in many patients with common variable hypogammaglobulinemia who also have a persistence of B-lymphocytes and evidence for excessive "suppressor T-cell" activity.

Further studies of the nature of this phenomenon have shown that suppressor T-cells develop in bursectomized birds after all Ig has disappeared from their circulation and that such suppressor T-cells have specificity for each of the immunoglobulin classes. Thus, when limiting members of T-cells from Bx agammaglobulinemic chichs are transplanted into normal recipients, some recipient birds develop deficiency of all classes of Ig while others develop dysgammaglobulinemia. This dysgammaglobulinemia is characterized by loss of one or more, but not all, classes of Ig. T-cells obtained from these dysgammaglobulinemic birds are then capable of transferring this class specific suppression to other normal birds but do not suppress Ig classes which were normal in the donor chicken. Thus, animals with selective deficiency of IgG, IgA, or IgM as well as birds with deficiency of IgG and IgA, or IgM and IgA, or IgM and IgG have been produced. This Ig class specific suppression also closely parallels various human immune deficiency diseases with deficiency in one or more classes of Ig and provides an excellent experimental model to further investigate the role of T-cell regulation of Ig production and potential therapeutic maneuvers to correct such deficiencies in man.

Humoral factors playing an important role in immunoregulation have also been extensively studied. One of the most striking examples of immunoregulation is found during pregnancy when the histocompatible fetus and placenta is protected from immunologic attack by the cells and immunoglobulins of the mother. Previously we have shown that human chorionic gonadotropin (HCG), a glycoprotein hormone produced in vast quantities by the syncitiotrophoblast of the placenta, contains potent immunosuppressive activity. This immunosuppression is demonstrable *in vitro* as a totally reversible inhibition of lymphocyte proliferation responses to nonspecific mitogens like PHA, to specific antigens like Candida, and to allogeneic cells in the mixed lymphocyte reaction. HCG also inhibits the ability of lymphocytes, PMN's and monocytes to kill cellular target cells in a variety of assays. Further studies, however, have shown that highly purified homogenous preparations of HCG do not manifest immunosuppressive activity. Evidence suggests that a small molecular weight compound present in the urine of pregnant women (but not non-pregnant individuals) which co-purified with HCG during its commercial preparation is responsible for the immunosuppressive activity. Other investigators have indicated that alpha-fetoprotein, another protein found in very high concentrations during pregnancy also has potent immunosuppressive properties. Our studies of the effect of highly purified alpha-fetoprotein obtained from an alpha-fetoprotein producing hepatoma have failed to confirm the potent immunosuppressive activity of this material. It is now becoming clear that compounds with the properties

of an almost ideal immunosuppressant (non-toxicity, reversibility, activity at low concentration) are present during pregnancy but that these (this) materials have not yet been convincingly identified. The identification and purification of this material will prove extremely useful for understanding mechanisms of immunoregulation and may potentially provide a potent compound for clinical control of immune responses.

Another striking example of immunoregulation is the failure of autoimmune reactivity in most individuals. Extensive studies in experimental animals and man have demonstrated an almost universal capacity for autoreaction which is normally held in check by unknown mechanisms. T-lymphocytes have long been known to react to foreign histocompatability antigens by proliferation in vitro (the mixed lymphocyte reaction - MLR). We have found that normal "T" lymphocytes also respond vigorously in vitro to "B" lymphocytes from the same individual, thus, giving an "autologous mixed lymphocyte reaction." Under appropriate tissue culture conditions, this auto-reactive MLR approaches the magnitude of the response observed in the more commonly studied allogeneic MLR. In studies of the factors which normally prevent such auto-reactivity from occurring in vivo, we observed that cortisol in physiologic concentrations (4×10^{-7}M) totally abolished this autologous MLR while having no effect on the "T" cell response in foreign histoincompatability cells in the allogeneic MLR. Even 20-fold higher cortisone concentrations only partially inhibit this allogeneic reaction. Thus, concentrations of cortisol found in normal human plasma have profound immunoregulatory effects selectively inhibiting autoimmune reactivity while not interferring with immune responsiveness to foreign tissue antigens.

Studies of the mechanism of action of various cytotoxic cells and their contribution to host defense were continued. These studies included evaluation of the capacity of immune lymphoid cells to destroy target cells bearing the immunizing antigen as well as model systems of the generation of cytotoxic cells utilizing mitogens as a cytotoxic cell activator or the use of target specific IgG antibodies as the "director" of cell mediated target lysis. Cellular immunity to measles (rubeola) was evaluated in patients with immunodeficiency disease and in patients with subacute sclerosing panenchephalitis (SSPE), a progressive central nervous system disease caused by measles virus. A new assay for cell mediated immunity to measles was developed which utilized a cell line chronically infected with an SSPE strain of measles virus as the target cell. Peripheral blood lymphocytes from normal immune individuals destroy these chronically measles infected target cells while lymphocytes from non-immune individuals are unable to do so. Lymphocytes from patients with chronic lymphocytic leukemia, the Sezary syndrome and various immunodeficiency disorders characterized by severe T-cell defects were unable to kill these target cells. Patients with immunodeficiency isolated to the humoral immune system had lymphocytes with normal cytotoxic potential for these measles infected target cells. Significantly, lymphocytes from patients with SSPE had enhanced killing of the measles virus infected target cells, disproving a proposed etiology of this disease (i.e., defective cellular immunity to the measles virus).

Further studies of cellular immunity to measles were carried out in hamsters. Within two days of infection of weanling hamsters with any of several strains of measles virus, strong cellular immunity could be demonstrated. This active cellular immunity persisted at a high level until 14 days after infection when serum antibody first appeared. As the antibody to measles rapidly increased in titer, cellular immunity wained to low levels. Reinfection of these animals with measles virus resulted in a brief elevation in cytotoxic lymphocyte activity but a long term elevation is serum neutralizing antibody.

Cells with cytotoxic potential can also be demonstrated by their ability to kill certain target cells in the presence of specific target cell directed IgG antibody. This antibody dependent cellular cytotoxicity (ADCC) reaction is dependent on the source of the antibody, the type of effector cell and the nature of the target cell. A special type of ADCC reaction utilizing human group B red blood cells as targets and human hyperimmune anti-B antibody was found to utilize monocytes and polymorphonuclear leukocytes as effector cells rather than lymphocytes as the killer cell. Utilizing this reaction as a clinical test of monocyte function we have observed several diseases with defective monocyte cytotoxic activity. The Wiskott-Aldrich syndrome is an x-linked recessive disease characterized by thrombocytopenia, eczema, and recurrent infections with all classes of microorganisms. Our studies have previously defined many defects in humoral and cellular immunity in boys with this disease. Although ADCC activity of the lymphocytes from these patients is normal, we found that monocyte mediated ADCC activity was virtually absent in these children. This abnormality appears to be an intrinsic defect in the cellular cytotoxic mechanism of these cells since they were found to have normal cel surface receptors capable of binding IgG coated erythrocytes and even were able to phagocytize these target cells normally. Similar studies have demonstrated monocyte cytotoxic defects in patients with chronic muto-cutaneous candidiasis, lymphohistocytosis and various childhood malignancies. Since the monocyte and macrophage plays a central role in mnay cellular immune reactions such as delayed hypersensitivity reactions and tumor rejection, such a defect in monocyte cytotoxic capacity helps to explain an element of the cellular immune deficiency in these patients.

Significance to Biomedical Research: The present studies extend our understanding of the diverse, yet interrelated mechanisms contributing to the development and normal functioning of immune system in animals and man. They indicate that disorders of immune function may be related to the absence of malfunction of such central lymphoid organs as the thymus or Bursa of Fabricius or to immaturity of such auxillary systems as the macrophage required for the processing of antigens in the initiation of an immune response. In addition, they indicate that immunoregulatory cells and humoral materials may have a profound effect on the function of the immune system both in health and disease. These studies have also provided new insights into some basic defects in accessory immune elements which provide new avenues for work designed to correct malfunctions in immunity in malignant and immuno-deficiency disorders.

Proposed Course: Continuing studies directed toward understanding the normal pathways involved in the development and function of the immune system and application of this new knowledge in approaches to the prevention and therapy of malignant disease.

Publications:

Poplack, D.G., Sher, N.A., Chaparas, S.D., and Blaese, R.M.: The effect of mycobacterium bovis (Bacillus Calmette-Guerin) on macrophage random migration, chemotaxis, and pinocytosis. Cancer Research 36: 1233-1237, 1976.

Kisli, I.R., Poplack, D.G. and Blaese, R.M.: A nonspecific esterase stain for the identification of monocytes and macrophages. In Methods in Cellular Immunology, B. Bloom and J. David, eds. Academic Press, 1976 In press.

Blaese, R.M.: Macrophage function in the development of immunocompetence and in immunodeficiency. J. RES. In press.

Muchmore, A.V., Nelson, D.L. and Blaese, R.M.: The cytotoxic effector potential of some common non-lymphoid tumors and cultured cell lines with phytohemagglutinin and heterologous anti-erythrocyte antisera. J. Immunol. 114: 1001-1003, 1975.

Muchmore, A.V., Nelson, D.L., Kirshner, H., and Blaese, R.M.: A reappraisal of the effector cells mediating mitogen induced cellular cytotoxicity. Cellular Immunol. 19: 78-90, 1975.

Muchmore, A.V., Koski, I., Dooley, N., and Blaese, R.M.: Artifactual plaque formation in vitro and in vivo due to passive transfer of specific antibody. J. Immunol. In press.

Blattner, W., Strober, W., Muchmore, A.V., Blaese, R.M., Broder, S., and Fraumeni, J.F.: Familial chronic lymphocytic leukemia: Immunologic and cellular characterization. Ann. Int. Med. In press.

Blaese, R.M., Muchmore, A.V. and Nelson, D.L.: Cellular cytotoxicity reactions induced by mitogens. In Mitogens in Immunobiology, Rosenstreich, D. and Oppenheim, J.J., eds. Academic Press, 1976. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4016(c)-02-MET

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanism of the Anabolic Action of Growth Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Peter Nissley, M.D.	Senior Investigator	MET	NCI
OTHER:	Alan C. Moses, M.D.	Clinical Associate	MET	NCI

COOPERATING UNITS (if any)

Diabetes Branch, NIAMDD

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

A prerequisite to understanding malignant growth is understanding normal growth. A growth hormone treated hypophysectomized animal has served as one experimental model. It has been proposed that the anabolic action of growth hormone is mediated by a second circulating protein designated somatomedin.

PHS-6040
(10-75)

Project Description:

Objectives: The understanding of normal growth may be prerequisite to understanding malignant growth. One experimental model for the study of normal growth has been the growth hormone treated hypophysectomized animal. It has been proposed that the anabolic action of growth hormone is mediated by a second circulating protein designated somatomedin. The object of this project is to purify somatomedin, develop an in vitro tissue culture system responsive to somatomedin and study the biochemical events accompanying growth in the system.

Methods Employed: Somatomedin is being purified from the conditioned medium of rat liver cells in culture. This rat liver cell line grows in the absence of serum and produces a growth factor identical to or closely related to somatomedin (multiplication stimulating activity of MSA). Serum starved cells in culture are being utilized to study the action of purified MSA. DNA synthesis is being measured by thymidine incorporation into DNA, flow microfluorometry and cell multiplication.

Major Findings: In collaboration with Matthew Rechler, Diabetes Branch, NIAMDD, we have demonstrated a receptor for multiplication stimulating activity (MSA) on the surface of various cells in culture (chick embryo fibroblasts human skin fibroblasts, and Buffalo rat liver cells). In the case of the chick embryo fibroblast receptor, ability of unlabelled MSA, insulin or somatomedin A to displace ^{125}I-MSA from the receptor correlated with the potency of these peptides in stimulating DNA synthesis and growth of chick embryo fibroblasts. The concentration of MSA, insulin or somatomedin A required for half maximal stimulation of [^{3}H] thymidine incorporation into DNA. We, therefore, believe that MSA binding to chick embryo fibroblasts defines a growth receptor. Using the above chick embryo fibroblast system it has been shown that proinsulin is 10-20 fold more potent in interacting with growth receptor and stimulating DNA synthesis than expected from proinsulin's potency in in vitro assays for insulin-like metabolic activity. These experiments suggest that insulin and proinsulin exhibit growth promoting effects on cells in culture via a mechanism different than the pathway for the classic metabolic effects of insulin.

Skin fibroblasts have been cultured from 4 growth hormone deficient patients and serum obtained from those patients before and after growth hormone (GH) treatment has been compared for ability to stimulate DNA synthesis and growth in autologous skin fibroblasts. The skin fibroblasts grew equally well in the pre- and post-GH sera suggesting that macromolecules which are required for the growth of human skin fibroblasts in culture are not growth hormone dependent. In contrast, chick embryo fibroblasts grew better in media containing an extract of post-GH human serum than in media containing an extract of the pre-GH serum.

Significance to Biomedical Research: The understanding of the biochemical events accompanying normal growth should provide a groundwork for understanding the aberations in growth accompanying malignancy. Also, further

delineation of the hormonally dependent growth factors necessary for the growth of cells in culture is of general importance in tissue culture, a widely utilized technique in cancer research. Specifically this research supports objective 3, approaches 1 and 2 and objective 4, approach 1 of the National Cancer Plan.

Proposed Course: In collaboration with Steven Rosenberg, Surgery Branch, DCT, we are going to test the hypothesis that the growth of human osteosarcoma may be growth hormone dependent. This will be tested by examining the growth of human osteosarcoma cells in pre- and post-GH sera from growth hormone deficient patients. We will also check the recent report that patients with osteosarcoma have elevated somatomedin levels.

MSA, which has been purified to homogeneity will be further characterized. The amino acid composition and terminal sequencing will be carried out in collaboration with Bryan Brewer, NHLI.

A radioimmunoassay for MSA is being developed and together with the radioreceptor assays will be utilized to measure MSA levels in the rat in various growth hormone states.

Publications:

Cohen, K.L., and Nissley, S.P.: Comparison of somatomedin activity in rat serum and lymph. Endocrinology. 97:654, 1975.

Megyesi, K., Kahn, C.R., Roth, J., Neville, D.M., Nissley, S.P., Humbel, R., and Froesch, E.R.: The NSILA-s receptor in liver plasma membranes: Characterization and comparison with the insulin receptor. J. Biol. Chem. 250:8990, 1975.

Rechler, M.M., Podskalny, J.M., and S.P. Nissley: Interaction of multiplication-stimulating activity with chick embryo fibroblasts demonstrates a growth receptor. Nature 259:134, 1976.

Nissley, S.P., Passamani, J. and Short, P.A.: Stimulation of DNA synthesis, cell multiplication and ornithine decarboxylase in 3T3 cells by multiplication stimulating activity (MSA). J. Cellular Physiol. In press.

Cohen, K.L. and Nissley, S.P.: The serum half-life of somatomedin activity: Evidence for growth hormone dependence. Acta. Endocrinologica. In press.

SUMMARY REPORT
LABORATORY OF PATHOLOGY
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
July 1, 1975 to June 30, 1976

The diagnostic and research activities of the staff of the Laboratory of Pathology are listed by Sections. The research accomplishments are especially impressive when one considers that the majority of the staff participate in the diagnostic services for Clinical Center patients. In fact, because of changes in professional personnel, including resignations and retirements of some staff members, and because of additional administrative responsibilities of others, the overall diagnostic workload of several members of the staff has increased appreciably during the past year. Dr. A.S. Rabson, Deputy Chief of the Laboratory of Pathology, was appointed Director of DCBD in April 1975. Later in the year he was given a dual appointment as Staff Pathologist in the Viral Oncology and Molecular Pathology Section. Dr. T.A.S. Hanson joined the staff as an Expert Pathologist in September 1975 and was assigned responsibility for the diagnostic services of the Surgical Pathology and Postmortem Section. He has made major changes in the organization of the professional and technical staff to improve these diagnostic services. Dr. Ralph D. Powell, Jr. resigned to take a position at the University of Kentucky (V.A. Hospital) in January, 1976. It is by offering opportunities and facilities for laboratory research that we have been able to recruit and retain an outstanding group of anatomical pathologists who have given vital support to the clinical programs of NCI as well as all the other Institutes with patients in the Clinical Center.

The research programs of the Laboratory have involved:

A. Hematopathology and Immunopathology
B. Viral Oncology and Cell Biology
C. Comparative Pathology and Carcinogenesis
D. Development and Use of Automated Equipment for Cytology Diagnoses
E. Clinico-Pathologic Studies on a Variety of Human Diseases

Some of the significant research and clinico-pathologic accomplishments in which the staff of the Laboratory participated, either as principal investigators or as collaborators include:

(1) Further characterization of the morphology, immunoglobulin production and surface membrane receptors on the cells of human malignant lymphomas and leukemias and relating these to similar studies of the normal cells of origin of these lymphomas and leukemias.

(2) Characterization of the pathogenesis of hepatic fibrosis and the development of angiosarcomas in the liver of workers exposed to vinyl chloride.

(3) Demonstration that propidium iodide - fluorescein isothiocyanate staining shows definite promise as a bicolor-fluorescent system for the automation of cytology screening of cervico-vaginal specimens.

(4) Differentiation of neurovirulent from attenuated strains of Type 1 polioviruses by the structural lability of virion polypeptides.

(5) Demonstration that a membrane Fc receptor which develops on the surface of cells infected with HSV is related to synthesis of early viral products. Engagement of this surface membrane receptor by IgG interferes with viral production. These observations may be important for elucidation of latency in viral infected cells.

Many members of the staff, in addition to their laboratory and service work, serve on numerous national and international committees, editorial boards, etc. Some of the principal activities of this sort are: Dr. C. Dawe, Project Officer for the Registry of Tumors in Lower Animals (Smithsonian Institution); Dr. C. Herman, Co-chairman of the DCBD Committee on Cytology Automation; Dr. L.B. Thomas, WHO Committee on Classification of Tumors of the Female Genital Tract and WHO Committee to develop an International Classification of Diseases - Oncology (ICD-O) and Council Member of International Academy of Pathology (USA-Canadian Division); Dr. C. Berard, Pathology Editor of the Journal of the RES, Associate Editor of Laboratory Investigation and member of the USA Lymphoma Panel; Drs. C. Berard, E. Jaffe and R. Braylan on faculty of Tutorial on Neoplastic Hematology; Dr. D. Henson, Short Course on Pathology of Virus Infections, IAP and member of College of American Pathology Committee to develop the Systematized Nomenclature of Medicine (SNOMed); Dr. Thomas, Dr. Rabson and staff, lecture series in pathology to Georgetown University medical students; Dr. E. Chu, faculty of Aspiration Cytology Workshop, American Society of Cytology.

Dr. José Costa was transferred from the Surgical Pathology Section to the Section on Viral Oncology and Molecular Pathology. Dr. A.J. Garvin completed residency training in June, 1976 and accepted a position as Staff Pathologist to replace Dr. Powell in the Surgical Pathology and Postmortem Service. He will be on assignment working with Dr. E. Neufeld during 1976-1977 but will spend part-time in Surgical Pathology and will set up a histochemical laboratory for the Surgical Pathology and Postmortem Section. Dr. Colin Wood, Professor of Pathology and Head of Surgical Pathology at the University of Maryland Medical School, will spend a sabbatical year working in Surgical Pathology with Dr. Hanson during 1976-1977.

Dr. Risa Mann, from Johns Hopkins University School of Medicine, started working with Dr. Berard as a Staff Fellow (Commissioned Officer) in July, 1975 and will continue in this appointment through June of 1977. In addition, Dr. Berard and his staff are working with 3 other guest workers and visiting scientists: Dr. Koji Nanba from the University of Hiroshima

(International Research Fellow-Fogarty, July, 1974 - September, 1976); Dr. Shaw Watanabe (Guest Worker/Visiting Scientist, June, 1975 - May, 1976) from Keio University School of Medicine, Tokyo, Japan, and Dr. Marie Ricard from Centre Hospitalier et University de Creteil, France (Guest Worker, October, 1975 - September, 1976). Dr. Go Akagi of Tokushima University, Japan (Guest Worker, September, 1975 - September, 1976) is working with Dr. Stanton. Dr. Yuji Kurokawa from Tohoku University, Sendai, Japan is working with Dr. Dawe as a Fogarty International Research Fellow (Guest Worker, October, 1975 - October, 1976). Dr. Oliver Alabaster has an Expert position and is working with Dr. Herman in the Quantitative Cytology Section. Dr. Ann T. Cheng is a Visiting Fellow (November, 1975 - November, 1976) working with Dr. Chu. Dr. Mary Proctor (PHS Research Fellowship, NIAID, August, 1975 - August, 1976) is working with Dr. Young.

During 1976-1977 several other Guest Workers or Visiting Scientists may work with the staff in the Laboratory of Pathology, depending on approval of support funds.

The staff were either senior authors or coauthors of 37 papers and 8 abstracts published in 1975. Four papers have been published through March of 1976 and there are currently 69 papers in press or submitted for publication.

Surgical Pathology and Postmortem Section

Dr. T.A.S. Hanson together with the residents and other pathologists from the Laboratory of Pathology plus Dr. R. Friedman (NIAMDD), Dr. A. Cheever (NIAID), Dr. K. Stromberg (NCI), and Dr. W. Roberts (NHLI) were responsible for the surgical pathology and autopsy diagnostic services. Approximately 7300 surgical specimens or biopsies were accessioned during 1975; 2526 were bone marrows. 293 autopsies were accessioned; 119 of these were submitted. These specimens involved the preparation of more than 64,000 slides including nearly 44,000 H & E stained sections, more than 8,000 special stains and 485 frozen sections.

Many specimens of fresh human tissues, including the eyes which are regularly removed during a complete autopsy, are furnished to NIH scientists in various laboratories.

Conferences with individual clinicians and surgeons from the various institutes are an important part of the care and study of patients in the Clinical Center. Regular gross specimen reviews of organs removed at autopsy are conducted by the staff and most surgical specimens are discussed at the weekly Surgical Pathology Conference conducted by Dr. Hanson and the residents and staff. In addition there are other regular conferences including the Hematology-Lymphoma Conference and the Lymphoma Staging Conference (Drs. Berard, Jaffe and Braylan), the Pediatric-Oncology Conference (Dr. Triche), and a monthly Neuropathology Conference (Dr. Valsamis). The staff and residents present selected autopsies at numerous clinico-pathologic conferences (e.g. NCI - Dr. Hanson, NHLI - Dr. Roberts).

During the year Dr. Hanson spent a lot of time and effort re-organizing the diagnostic services. Major improvements included careful and prompt reporting of all surgical pathology reports. Also, the autopsy service is now operating more efficiently and prompt completion of autopsy reports within 4-6 weeks of the date of autopsy is regularly achieved. With the help of Mrs. Josephine Nelson who worked temporarily for 2-3 months, the several years of backlog departmental copies of surgical pathology and autopsy reports were sent to the bindery. Bound volumes of these reports are now current through 1975 and greatly improve the accessibility of records for study and review. Dr. Hanson has also developed plans to re-institute coding of pathology diagnosis to bring the diagnostic file up to date and to coordinate this diagnostic file with the computer-based communication reporting system which is being installed in the Clinical Center.

Publications of Studies on Human Material

More than half of the 1975 publications by the staff and residents were based on human tissues or some aspect of human disease. These include: vinyl chloride-induced hepatic angiosarcoma and fibrosis (Thomas - 1,10,11); technical errors in histological preparation of lymph nodes (Berard and bowling - 3); mycosis fungoides (Thomas - 19); malignant lymphomas and neoplasms of the reticuloendothelial system including Burkitt's tumor and hairy cell leukemia (Berard - 5; Banks and Berard - 8,9; Jaffe and Berard - 6,15; Mann, Jaffe, Braylan and Berard - 7; Braylan, Jaffe and Berard - 25; Braylan - 27,28,29); seminal vesicle cyst containing mesonephroid tumor (Witebsky and Thomas - 12); cytopathology of human tumors (Chu and staff - 14); quantitative cytology on vaginal-cervical cytology samples (Cassidy, Fowlkes and Herman - 16); viruses and the etiology of human hematopoietic neoplasms (Rabson and Costa - 17); pneumatosis coli in cystic fibrosis (Herman - 24); binding sites for various lectins on human erythrocyte membranes (Triche - 26); parathyroid lesions (Costa - 30); Anitschkow nuclear changes in oral epithelium (Wood, DeWitt, Chu and Rabson - 32); ectopic and normal alpha subunits of human glycoprotein hormones (Rabson - 33); preparing lymphocytes for SEM (Sanders and Braylan - 34); cytology of bronchial washings (Chu - 37). Reference numbers are to the attached list of 1975 publications.

There are a number of other on-going studies of human disease. Dr. Thomas, Dr. Popper and Dr. Telles are continuing a major review of hepatic angiosarcomas known to be due to vinyl chloride, arsenic or thorotrast and a group of angiosarcomas without known etiology. The epidemiologic aspects of this review are conducted by staff (Drs. John Herbert and H. Falk) of the CDC. Dr. Rabson and Dr. N. Telles have completed a study of the pathologic findings in patients with Ewing's sarcoma. Dr. Thomas and Dr. Hanson with Dr. L. Ackerman and Dr. Robert McDivitt have started a program to review approximately 600 breast cancers which were accumulated during the past 10 years under the HIP study. This review will be coordinated with reviews of the epidemiologic and radiologic risk/benefit factors for mammography as a screening technique for breast cancer. Dr. Thomas, Dr. D. Dahlin, Dr. Rabson and Dr. J. Kolins are reviewing the Stockholm cases of osteosarcoma treated with interferon. Other projects

include study of pulmonary candida infections (Dr. Hanson and Dr. Young), evaluation of adrenal and renal changes in hyperaldosteronism (Dr. Orlando), agnogenic myeloid metaplasia (Dr. Orlando), and a review of approximately 600 non-Hodgkin's lymphomas (Dr. Garvin and Dr. Berard). Dr. Robert Stern has an especially active research program supported by his laboratory in NIDR. Included in his projects are several studies on the biochemical characteristics of collagen associated with various human diseases.

Miss Bowling and her staff continue to provide excellent histological slides and special stains for human tissues. She is also a valuable consultant on histologic techniques to numerous NIH scientists and Clinical Center physicians.

Photographic services for human tissues are provided by Mr. K. Edwards, Mr. Banks and Mr. Isenburg. Mr. Banks also maintains the slide files which now contain well over a million slides. The photographic laboratory is also used by numerous Laboratory of Pathology investigators and technicians, especially by those who develop and process photomicrographs for E.M.

Cytopathology Section

Dr. Chu and her staff provide complete diagnostic service in exfoliative cytology, medical cytogenetics and fine needle aspiration cytology. Chromosomal analysis includes standard karyotyping and special techniques using trypsin digestion and G-banding for the identification of individual chromosome pairs. During the year there were more than 4,000 cytology specimens accessioned. Approximately 1/3 of these were vaginal and cervical specimens and nearly two-thirds (2,772) were other types of specimens including ascitic, pleural, gastric, prostatic, spinal fluid, bronchial specimens and wound washings. Approximately one-half of these specimens were from NCI patients and slightly more than half of the specimens were from the Clinical Center patients of 10 of the other institutes.

Quantitative Cytology Section

Dr. Herman was appointed Head of this newly created Section during the year. He and his staff (including Dr. Bunnag, Dr. Alabaster, Ms. Cassidy and Ms. Fowlkes) are responsible both for the intramural research program and for supervision of the extramural contracts under the DCBD Cytology Automation Program. During the year they completed preliminary evaluation of propidium iodide-fluorescein isothiocyanate staining as a bicolor fluorescent system for screening gynecologic cytology specimens (Ms. Fowlkes). This system is one of but two or three systems which show definite promise for the automation of cytology screening. Other projects completed included: using flow microfluorometric life cycle analysis to distinguish in vivo resistance or sensitivity of L1210 ascites cells to a chemotherapeutic agent, cytosine arabinoside, (Drs. Alabaster and Bunnag); demonstration that a hematoporphyrin derivative lacks suitable sensitivity and specificity as a marker to distinguish premalignant and malignant changes in cytologic specimens (Ms. Cassidy); and demonstration that flow microfluorometric determination of lymphocyte response to H. virus antigen is

sensitive and suitable as an assay technique (Ms. Cassidy in collaboration with Dr. Costa and Ms. Yee). These are very impressive accomplishments by Dr. Herman and his staff, particularly when one considers that much effort and time have been expended during the past 2 or 3 years getting complex cell sorters and other equipment operational and that for the past 2 years Dr. Herman has also served as a full-time resident in Clinical Pathology. His plans during the coming year include major efforts to develop and evaluate stoichiometric fluorescent cytochemical stains for fluidic analysis and sorting of cells on the basis of cell content of histones, non-histone chromosomal proteins, cytoplasmic S-H groups and cellular levels of enzyme activity. Major effort will also be made to develop algorithms for analysis of multiparametric flow system data. Pharmacokinetic studies on the effects of chemotherapeutic agents to alter tumor cell life cycles will be continued. Similar flow microfluorometric analysis techniques will also be used to study glucocorticoid effects on human lymphoblasts and on the multiplication and stimulating activity of a variety of substances on human, mouse and chick fibroblasts. Other projects include study of biochemical and biophysical parameters of malignant cells of hematopoietic tissues (with Dr. Braylan) and of breast cancer culture lines (with Drs. Lippman, Young and Ms. Engel). A research program to analyze and sort defined chromosomes of one species is being initiated to study the results of introducing these chromosomes into host cells of another species (with Dr. McBride, Laboratory of Biochemistry).

Ultrastructural Pathology Section

Dr. Timothy J. Triche joined the Laboratory of Pathology staff in July, 1974. In less than 2 years he has organized and is operating a very productive program involving transmission and scanning E.M. and freeze-etch electron microscopy. During the year he and the staff of the Section processed and studied approximately 176 human tissue specimens for diagnostic and/or investigative electron microscopy. In collaboration with Dr. Shaw Watanabe all of the submitted lymphoma cases for 1975 as well as specimens from 1974 and from 1976 will be examined by E.M. This is an important aspect of the overall study of the cells, the malignant lymphomas being conducted by Drs. Berard, Jaffe, Braylan and their guest workers and visiting scientists. Dr. Triche has organized the laboratory so that Mr. Michelitch is primarily responsible for the processing and initial screening of diagnostic E.M. specimens with the result that many of these studies are completed within a few days time of receipt of the specimen. Two of the residents, Dr. Reddick and Dr. Garvin, have been especially involved with E.M. study of current human tissue specimens. Ms. Mims has sole responsibility for technical support in the many collaborative research projects. These include: study of interferon inhibition of MLV replication (with Dr. Friedman); effect of streptozotocin on glucagon secreting tumor cells; and characterization, isolation and distribution of IgE-specific Fc receptors on rat basophil leukemia cells.

Dr. Triche has developed plans for a remodeled laboratory in space on the 2A corridor to house the transmission and freeze-etch electron microscopes. This change will produce a major effect on both his research and diagnostic

work because it will place him and his staff in close proximity with the other laboratories handling human tissues from the Surgical Pathology, Postmortem and Cytology Sections. Dr. Triche is a skilled diagnostic pathologist and participates in all phases of the diagnostic services including review of surgicals and autopsies with residents.

Dr. Donald Henson has continued his experimental work to develop immunohistochemical methods using immunofluorescence and immunoperoxidase techniques for the detection of tumor markers such as myosin in rhabdomyosarcomas and other sarcomas in tissue sections and cell suspensions. Experiments on chronic CMV infection are continuing. During the year he has also made a significant contribution to the WHO Committee developing ICD-O and to the College of American Pathology Committee developing SNOMed. Dr. Henson participates in the pathology diagnostic services and residency training program.

Hematopathology Section

Drs. Berard, Jaffe, Braylan and their co-workers Dr. R. Mann, Dr. Nanba, Dr. Watanabe and Dr. M. Ricard have major ongoing programs in diagnostic and experimental studies of neoplastic and non-neoplastic lesions of the lymphoreticular and hematopoietic systems. Dr. Berard has an enormous referral of cases of malignant lymphomas; nearly 650 cases were reviewed and reports prepared during the past year. Of these 225 were from the cooperating institutions in the Eastern Cooperative Oncology Group and 418 were submitted for consultation and diagnosis from pathologists in the USA and foreign countries. In addition, Dr. Berard and the staff were responsible for reviewing all of the cases of malignant lymphoma and related conditions admitted to the Clinical Center. Fresh biopsy tissue was usually available for the latter cases and this was used by Dr. Jaffe, Dr. Braylan and others on the staff for special immunologic, histochemical, cytochemical and ultrastructural studies. This is a very productive program as indicated by the relatively large number of publications during the past year (see attached list of publications). Included were studies on the immunologic and morphologic features of a T-cell lymphoma, American Burkitt's tumor, cases of "hairy cell" leukemia, the Sezary cell and various other malignant lymphomas and leukemias. The results of these studies continue to add new information about the functional characteristics of the cells of these neoplasms and their relationship to the normal cells from which they originate.

Drs. Berard, Jaffe and Braylan were each invited to give several scientific lectures at scientific meetings and continued to serve as faculty members for the Eighth Tutorial on Neoplastic Hematology at the University of Chicago.

Dr. Berard served as co-chairman for planning a retrospective review of approximately 1000 cases of malignant lymphoma. He will be a contract officer and one of the team of pathologists to review these cases at various centers in the USA and perhaps in other countries in order to compare and correlate several of the published classification schemes for the non-Hodgkin's malignant lymphomas.

Drs. Berard, Braylan and Jaffe participate in the diagnostic services of the laboratory and conduct several clinico-pathologic conferences. In addition, Dr. Jaffe serves as chairman of the residency selection program. Dr. Risa Mann also participates in the diagnostic services of the laboratory, reviews ECOG and consultation cases and is involved with several of the special studies on the malignant lymphomas.

Comparative Oncology Section

Drs. Dawe, Banfield and Stanton are the professional staff members of this Section. Dr. Dawe's studies on polyoma virus transformation of mouse salivary gland epithelium in cell and organ cultures have continued. In trans-filter experiments he has found that neoplastic transformation can be induced by PV in either epithelium or in mesenchyme when the two tissues are not interacting normally by direct cell to cell contact. Also the tumors resulting from such experiments are unusual in that they are highly undifferentiated, do not resemble the salivary gland tumors induced by PV in intact salivary glands and sometimes showed differentiation of unexpected type, including chondrosarcoma and osteosarcoma. In collaboration with Dr. Banfield and Dr. Sonstegard he has studied 2 different types of intracytoplasmic bodies in the lymphoma cells of the northern pike. The "cylindroid lamella-particle complex is of particular interest because similar structures have been found in neoplastic cells of human CLL, lymphosarcoma cell leukemia, "hairy cell" leukemia, monocytic leukemia and Waldenstrom's macroglobulinemia. The significance and function of these bodies and the other cytoplasmic structure, the "nucleoid body", are not known. Dr. Dawe has initiated experiments, in collaboration with Dr. Orlando, to study PV induction of mammary gland tumors in athymic (nude) mice. The pertinent question to be answered from these experiments is whether such induced tumors arise from hyperplastic alveolar nodules or from other parts of the duct epithelium. Dr. Kurokawa, an NIH Fellow, is working with Dr. Dawe studying the epithelial-mesenchymal interactions of gastric carcinoma induced by MNNG. Mr. Morgan uses the time-lapse motion picture equipment to study epithelial-mesenchyme interactions in various experiments. He has observed periodic synchronous contractions of the acinar portions of salivary glands in organ cultures. His observations of this phenomenon, which has not been previously reported, suggests that the network of myoepithelial cells contract as a unit similar to the contractions of cardiac muscle.

Dr. Stanton has been joined by Dr. Go Akagi of Tokushima University, Japan, this year and they are studying ultrastructural changes in respiratory epithelium preceding the development of chemically induced epidermoid carcinoma. Dr. Stanton has continued his major study of the critical role of fiber size in carcinogenesis. Earlier work with other fibrous materials has been confirmed in experiments with different sized sodium aluminum carbonate crystals in that these crystals also prove to be highly carcinogenic only in specific size ranges. Dr. Stanton also reports that the establishment of fibrous tremolite as a carcinogen has created considerable cancers because of potential environmental exposure. Dr. Stanton is currently planning the renovation of new laboratory space on the 8th floor for his research. In addition to his research, Dr. Stanton

is the project officer for 3 contracts, the largest of which is the long-term laboratory animal holding contract with Pharmacopathics, Inc.

Dr. Banfield has collaborated with Dr. Dawe to study the ultrastructure of the "cylindroid-lamella complex" bodies observed in lymphoma cells and reported his findings at a workshop on the northern pike lymphoma held in Stockholm in October, 1975. The pike lymphoma is the only known animal tumor affording the opportunity to investigate these structures which are of particular interest because similar structures have been found in some human lymphomas and leukemias. Other projects for which Dr. Banfield conducted E.M. studies were the study of joint tissues in collaboration with Dr. Hough and Dr. Sokoloff and on the activation of plasma membrane ectoenzymes by treatment with neuraminidase in collaboration with Drs. Trams and Lauter.

Viral Oncology and Molecular Pathology Section

Dr. Nathaniel Young has several major research projects on the polypeptides of polioviruses and on the possible role of oncornaviruses in the etiology of human breast cancer. Dr. M.E. Proctor, a Postdoctoral Fellow, and Dr. James A. Strauchen, a Clinical Associate assigned to Dr. Young's laboratory, have participated in these studies. He has demonstrated that the structural lability of the largest virion polypeptide (VP1) of type 1 vaccine strains can be used as a marker to differentiate neurovirulent from attenuated strains of poliovirus. Six strains received as coded unknowns from the CDC were correctly identified as of vaccine or wild origin. Further studies are being conducted to elucidate whether "leakiness" (backmutation to properties associated with virulence) can be detected in type 1 polioviruses isolated serially from the stools of children who have been fed vaccine. The significance of these findings is that protein analysis may have potential in determining whether human paralytic poliomyelitis following vaccine administration is caused by the vaccine virus or by concomitant infection with naturally-circulating neurovirulent strains. Dr. Young and his co-workers have succeeded in establishing 3 culture lines of human breast cancer and these have been used to characterize the cells biologically and to search for evidence of oncornavirus expression. Major findings from one of the culture lines include: observation of stable karyotypic abnormalities; ultrastructural features compatible with secretory epithelial cells; synthesis of differentiated proteins such as alpha lactalbumin and casein; presence of receptors for estrogen and other steroid hormones; and growth responsiveness to estrogen and insulin. No complete (infectious) oncornaviruses have been detected.

Dr. José Costa transferred from Surgical Pathology to the VOMP Section during the year and Dr. Rabson has continued an active research program in viral oncology in addition to his duties as Director of DCBD. The general scope of Dr. Costa's and Dr. Rabson's research is to study the biological properties of human herpesvirus and papovaviruses tentatively associated with human tumors. Studies are in progress to elucidate the significance of a binding site for the Fc fragment of IgG appearing on herpes simplex infected cells. The results suggest that the appearance of the Fc binding

site requires the synthesis of early products (pre-viral DNA synthesis) and that it probably is a set of viral coded antigens. It has also been demonstrated that engagement of the binding site with rabbit IgG and with Fc fragments of IgG interferes with viral production. These observations may represent a new approach to the understanding of herpesvirus latency. In collaboration with Dr. Triche, studies are being conducted to localize and characterize both the viral-associated membrane sites and lectin receptor sites on these HSV infected cells. A second major research project involves study of the oncogenic potential of MM virus, a human papovavirus isolate. In hamsters, this virus at the dosage used, produces ependymomas when injected intracranially but is not oncogenic when injected subcutaneously or intravenously. To date human lines derived from CNS tumors and 100 sera from cancer patients have been tested for the presence of papova-T antigen and no reactivity has been found.

Pathological Technology Section

This report represents the first full year of operation with half of the technical staff in Frederick and half of the staff in Bldg. 37. The 2 groups together processed more than 51,000 pieces of animal tissue (approximately 43,000 H & E and 3,500 special stained slides) for 87 different NCI investigators. This represents outstanding performance and productivity and provides a major type of laboratory service and support for a large segment of the research scientists in the NCI. The training of contractor personnel in various histologic techniques has continued and an electron microscope laboratory for animal tissues has been installed at Frederick. Mrs. Barbara Coolidge has served as Acting Head of this laboratory section since the retirement and death of Miss Betty Sanders in the summer of 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00853-23 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Surgical Pathology and Postmortem Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T.A.S. Hanson Medical Officer LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Surgical Pathology and Postmortem Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20	20	0

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgical Pathology and Postmortem Section, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The Staff of the Surgical Pathology and Postmortem Section also carries on independent and collaborative research.

PHS-6040
(12-75)

Project No. Z01 CBD 00853-23 LP
1. Laboratory of Pathology
2. Surgical Pathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1975 through June 30, 1976

Project Title: The Surgical Pathology and Postmortem Sections, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The Staff of the Surgical Pathology and Postmortem Sections also carries on independent and collaborative research.

Staff:

Dr. Thomas A. S. Hanson, Surgical Pathology and Postmortem Sections
Dr. Louis B. Thomas, Chief, Laboratory of Pathology
Dr. Jose Costa, Staff Pathologist
Dr. Costan W. Berard, Head, Hematopathology Section
Dr. Raul C. Braylan, Staff Pathologist
Dr. Clyde Dawe, Staff Pathologist
Dr. Donald E. Henson, Staff Pathologist
Dr. Elaine S. Jaffe, Staff Pathologist
Dr. Timothy Triche, Staff Pathologist
Dr. Nathaniel Young, Staff Pathologist
+Dr. A. Julian Garvin, Resident
+Dr. Peter M. Howley, Resident
+Dr. Micheal M. Orlando, Resident
+Dr. Robert L. Reddick, Resident
+Dr. Robert Stern, Resident
+Dr. Maria M. Tomaszewski, Resident
*Dr. Allen W. Cheever, National Institute of Allergy and Infectious Diseases
*Dr. Robert M. Friedman, National Institute of Arthritis, Metabolism, and Digestive Diseases
*Dr. William C. Roberts, Chief, Laboratory of Pathology, National Heart and Lung Institute
*Dr. Marius Valsamis, Associate Professor of Pathology, New York Medical College, Center for Aging and Chronic Diseases

+ - These physicians are full time Residents in the Laboratory of Pathology.
* - These Associate Pathologists spend part time in the activities of the Surgical Pathology and Postmortem Sections.

Project Description:

Objectives: The objectives of the Surgical Pathology and Postmortem Sections are: (1) to provide diagnostic services in pathologic anatomy; (2) to collaborate with National Institutes of Health investigators in research involving use and study of human material; (3) to conduct a residency training program in pathologic anatomy, and (4) to carry out independent research.

1. The service functions of the Sections during the period April 1975 through March 1976 included:

(a) 290 autopsy examinations. Included in this report is a table which shows the number of autopsies performed for each Institute. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Sections participate.

(b) 7546 specimens were accessioned in the Surgical Pathology Section. A complete listing of these specimens, by Institute, is given in the accompanying table. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissue removed for specific research projects.

(c) Histological preparation and special procedures:

These functions are carried out under the direction of Miss Mary C. Bowling and a complete listing of specimens processed by Institutes is given in the accompanying table.

(d) Photographic Services of the Department:

A photographic record is made of the large amount of the pathological material which is handled and studied in the department. The photography is done by the staff and residents with the technical assistance of Mr. L. Kenzie Edwards who also maintains the equipment and carries out much of the photographic processing. Gross photographs and photomicrographs of the pathologic material are provided to the clinical staff on request, and are used extensively for conferences, seminars and many are prepared for publication.

REPORT OF SURGICAL PATHOLOGY SECTION — April 1, 1975 through March 31, 1976

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMDD	NIAID	NINDS	NIMH	NIDR	NEI	NICH	REF	RESEARCH	TOTAL
Specimens accessioned	4383	336	206	269	107	10	658	4	41	1532	--	7546
Blocks of tissue cut	10419	557	648	471	337	48	432	2	186	1248	--	14448
Blocks of tissue recut	767	32	53	83	41	6	6	-	6	52	--	1046
Total blocks cut	11286	589	701	554	378	54	438	2	192	1300	--	15494
Slides made	33290	1776	2374	2335	942	183	937	8	529	6676	--	49050
Slides stained H&E	20807	975	1338	1274	498	92	470	4	261	6227	--	39146
Slides stained special	4759	232	322	564	90	14	19	-	68	394	--	6462
Total slides stained	25566	1207	1660	1838	588	106	489	4	329	6621	--	38408
Frozen sections	326	38	78	15	9	--	--	-	8	--	--	474

TOTAL SURGICAL AND POSTMORTEM PATHOLOGY SECTION REPORT:

Number of blocks cut ----------28685

Number of slides made ----------66429

Number of slides stained H&E ----------45778

Number of slides stained special ---------- 8279

Project No. Z01 CBD 00853-23 LP

REPORT OF POSTMORTEM PATHOLOGY SECTION

April 1, 1975 through March 31, 1976

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMDD	NIAID	NINDS	NIMH	NIDR	NEI	NICHD	REF	TOTAL
Autopsies performed	126	34	7	2	5	--	--	--	1	--	175
Autopsies submitted	12	3	1	1	--	1	--	1	1	95	115
Total autopsies	138	37	8	3	5	1	--	1	2	95	290
Blocks of tissue cut	9180	1869	421	158	268	58	--	42	53	511	12560
Blocks of tissue recut	452	55	28	15	81	--	--	--	--	--	631
Blocks serialled	--	--	--	--	--	--	--	--	--	--	---
Total blocks cut	9632	1924	449	173	349	58	--	42	53	511	13191
Slides made	11394	1865	528	195	422	58	--	57	53	2807	17379
Slides H&E stained	9392	1888	427	158	329	58	--	42	53	1485	13832
Slides special stained	847	154	58	23	75	--	--	15	--	645	1817
Total slides stained	10239	2042	485	181	404	58	--	57	53	2130	15649
Frozen sections	2	--	--	--	--	--	--	--	--	--	2

Listed below is a breakdown of the work performed by Mr. Edwards from April 1975 through March 1976:

Time taking photographs:	549 hours
Time processing:	249 hours, 30 minutes
No. of black and white negatives - 4 x 5 inches:	2901
Color transparencies - 4 x 5 inches - gross:	1666
35 mm. transparencies - gross:	9288
Total	10954
Prints contract out - 4 x 5 inches:	9092

A large proportion of the photomicrography is done under the direction of Mr. Ralph Isenburg who provides professional assistance and facilities for the entire staff of the Laboratory of Pathology.

2. The collaborative activities of the staff and residents in the Surgical Pathology and Postmortem Sections are reflected in the summary of research activities listed below:

(a) Distribution of human organs and tissues:

At present there are approximately 50 scientists in the various laboratories at the National Institues of Health who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

(b) Conferences:

The staff and residents take an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly organized solely or jointly by the department, including a Surgical Pathology Conference, Joint Pathologists Conference, Neuropathology Conference, Lymphoma Staging Conference, Medical Breast Cancer Service Pathology Conference, Medicine Branch Slide Review Conference, Pediatric Oncology Conference, Radiation Therapy Conference, Gastrointestinal Disease Conference, Brain Cutting Conference and Gross Autopsy Conference.

A. Dr. Thomas A. S. Hanson:

Entered on duty with the Laboratory of Pathology, September 2, 1975. Prior to joining our staff he was located at the University of Utah, College of Medicine and at the Veterans Administration Hospital in Salt Lake City, Utah. Since his arrival Dr. Hanson has addressed himself to removing a backlog of reports. At present, all reports are current.

B. A. Julian Garvin, M.D.

1. A retrospective study of all non-Hodgkin's lymphomas treated at NIH (approximately 600 cases) with reference to the current proposal classification, survival, progression of disease and therapeutic response.

2. A retrospective study of ovarian carcinomas treated at NIH with reference to their histologic type and grade.

3. A prospective study of lymphoreticular neoplasms using lysozyme as a cytochemical marker to determine their cell of origin.

Publications:

(a) Garvin, A.J., Pratt-Thomas, H.R., Williamson, Spector, M., and Spicer, S.S.: Gonadblastoma: Histologic, ultrastructural and histochemical observations in five cases. In Press.

(b) Garvin, A.J., Hall, B.J., and Spicer, S.S.: Cytochemical differentiation of nucleic acids with a Schiff-methylene blue sequence. In Press

(c) Parmley, R.T., Spicer, S.S., and Garvin, A.J.: Multilaminar endoplasm reticulum and abnormal mitoses in Hodgkin's tumor cells.

(d) McKeever, P.E., Garvin, A.J., and S.S. Spicer, Immune Complex Receptors on Cell Surfaces: I. Ultrastructural Demonstration on Macrophages. In Press

(e) McKeever, P.E., Garvin, A.J., Hardin, D.H., and S.S. Spicer, Immune Complex Receptors on Cell Surfaces, II. Cytochemical evaluation of their uptake, regeneration and relative abundance and distribution on different immune cells. Submitted.

(f) Garvin, A.J. and S.S. Spicer, The cytochemical demonstration of intracellular immunoglobulin: In normal and hyperplastic lymphoreticular tissue. Submitted

(g) Spear, G.S., Slusser, R.J., Garvin, A.J., Horger, E.O., Bailey, R.P., and schneider, J.A.: A cytoplasmic body in human fetal endothelium. Am. J. Path. 78: 333-342, 1975.

(h) Garvin, A.J.: Selective localization of immunoglobulin within normal hyperplastic and neoplastic lymphoreticular tissue. Ph.D. dissertation, Medical University of South Carolina, 1975.

(i) Spicer, S.S., Phifer, R.F., Garvin, A.J., and Zehr, D.: Immunostaining by an immunoglobulin bridge procedure. Presented at the International Symposium on Immunoenzymatic techniques. Paris, April 2-4, 1975.

(j) Garvin, A.J., Spicer, S.S., McKeever, P.E. The cytochemical demonstration of intracellular immunoglobulin: In neoplasms of lymphoreticular tissue. Am. J. Path. 82: 457-478, 1976.

C. Peter M. Howley, M.D.

1. Construction of a physical map of the human papovavirus BKV genome.

2. Characterization of the integrated viral DNA in BKV transformed hamster cells.

3. Characterization of the simian papovaviruses isolated from monkeys with PML.

4. Studies on DNA synthesis of human papovavirus BK.

5. Restriction endonuclease analysis of eukaryotic DNA.

6. Deveopment of a transcriptional map of BKV.

7. Morphologic studies on the endocrinologic changes in cystinosis.

8. Transformation of hamster and human cells with specific restriction endonuclease fragments of BKV DNA.

Publications:

(a) Howley, P.M., Mullarkey, M.F., Takemoto, K.K., and Martin, M.A.: Characterization of human papovavirus BK DNA. J. Virol. 15: 173-181, 1975.

(b) Khoury, G., Howley, P., Nathans, D., and Martin, M.A.: Post transcriptional selection of SV40-specific RNA. J. Virol. 15: 433-437, 1975.

(c) Khoury, G., Howley, P.M., Garon, C., Mullarkey, M.F., Takemoto, K.K., and Martin, M.A.: Homology and relationship between the genomes of papovaviruses, BKV and SV40. Proc. Nat. Acad. Sci. USA 72: 2563-2567, 1975.

(d) Howley, P.M., Khoury, G., Byrne, J.C., Takemoto, K.K., and Martin, M.A.: A physical map of the BKV genome. J. Virol. 16: 959-973, 1975.

(e) Khoury, G., Carter, B.J., Ferdinand, F.J., Howley, P.M., Brown, M., and Martin, M.A.: Genome localization of simian virus 40 RNA species. J. Virol. 17: 832-840, 1976.

D. Michael M. Orlando, M.D.

1. Evaluation of adrenal and renal histologic changes in patients with hyperaldosteronism.

2. Evaluation of renal pathology and comparison of pathologic findings with radiographic abnormalities in patients with agnogenic myeloid metaplasia.

3. Review of 500 slides of tissues from mice subjected to treatment with aromatic hydrocarbons.

4. Began study of morphologic characterization of development of mammary tumors in nude mice infected with polyoma virus.

Publications:

(a) Kwon-Chung, K.J., Young, R.C., Orlando, M., Pulmonary mucormycosis caused by Cunninghamella elegans in a patient with chronic myelogenous leukemia, Submitted to: Amer. J. of Clin. Path., 1975.

(b) Redlin, W.L., Orlando, M.M., Francis, R., Renal findings in agnogenic myeloid metaplasia, Submitted to: Diagnostic Radiology, 1975.

(c) Danforth, D.N., Orlando, M.M., Bartter, F.C., and Javadpour, N., Renal changes in primary aldosteronism, Submitted to: J. of Urology, 1975.

(d) Orlando, M.M., Chapman, G.B., and Panuska, J.A., Thyroid structure and function of rats treated with single and multiple doses of dimethyl-sulfoxide, Submitted.

(e) Orlando, M., Epstein, S., Herbert, P., Lipoprotein abnormalities in patients with angiographically demonstrated coronary artery disease. Submitted.

E. Robert L. Reddick, M.D.

1. Study of fine needle aspiration cytology of osteosarcoma and chondro-sarcoma in collaboration with Dr. Thomas A. S. Hanson and Elizabeth W. Chu.

Publications:

(a) Kramer, B., Hernandez, A., Levine, A., Reddick, R., Cutaneous mucor mycosis, Submitted.

(b) Kassin, S., Reddick, R., Lupus erythrematosus, In Press.

(c) Reddick, R., Hudson, P., Right ventricular aneurysm and rupture, In Press.

F. Robert Stern, M.D.

1. The nature of the desmoplastic reaction, in breast tumors with Dr. Nathaniel Young and Ms. Linda Engel.

2. The nature of the granuloma in Schistosomiasis: an animal model for fibrosis of the liver.

3. Clinical investigations reviewing material from this department with Dr. Jose Costa.

Publications:

(a) Benveniste, K., Wilczek, J., Ruggieri, A., and Stern, R.: Efficient translation of collagen messenger RNA in a cell-free system derived from wheat germ. In Press.

G. Maria M. Tomaszewski, M.D.

Publications:

(a) Tomaszewski, M., Henson, D.E., and Geelhoed, G.W.: Benign and second malignant primary neoplasms in women dying of breast cancer versus lymphoma. March 1975.

(b) Fishman, M.L., Tomaszewski, M., and Kuwabara, T.: Malignant melanoma of the skin metastatic to the eye-frequency in an autopsy series and relationship to metastatic behavior. Submitted.

(c) Cohen, M., Scardino, P., Tomaszewski, M.M.: Carcinoma arizing in a brachial cleft cyst, In Press.

(d) Cohen, M. and others, Tomaszewski, M.M., Prognostic factors in patients undergoing lymphadenectomy for malignant melanoma, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00504-20 LP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

An Electron Optical Investigation of Normal and Pathologic Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	C.J. Dawe	Medical Officer	LP NCI
	C.W. Lee	Biologist	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pathology

SECTION
Comparative Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

The transmission electron microscope is applied to significant problems directly or periferally related to neoplasia. The major effort over the past year has been a study of the cylindroid lamella-particle complexes in the cells of the malignant lymphoma of northern pike and its relation to a similar structure in human "hairy cell" leukemia and other human lymphomas and leukemias.

PHS-6040
(12-75)

Project Description:

Objectives: 1. To describe and interpret the ultrastructure of selected pathologic states and to compare the results with suitable controls.

2. To look for and study the inciting agent or accompanying agent in neoplastic and other disease states using human, animal and tissue culture material.

Major Findings: Two papers on the lymphoma of the northern pike have been submitted for publication. The work was presented and discussed at a workshop on the pike lymphoma held in Stockholm, Sweden in October.

Summary--Cells of the malignant lymphoma of northern pike of Canada contain a cylindrical structure with a central core of cytoplasmic elements and walls of lamellae in a spiraled arrangement separated by ribosome-like particles or membranes. These cylindroids are similar, if not identical to structures in the cells of certain human lymphomas and leukemias. In cross and longitudinal section the lamellae showed a pattern of light and dark bands resulting in a 95°A period which has not as yet been described in the human cylindroids. The pike lymphoma is the only presently known animal tumor affording opportunity to investigate these interesting structures.

Significance: A study of the cylindroid structure in the transmissible pike lymphoma when correlated with the studies on the similar structure found in human "hairy cell" leukemia may lead to a better understanding of the latter.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00593-21 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

An Investigation of the Collagenous Connective Tissues and Elastin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	L. Sokoloff	Medical Officer	State Univ. of N.Y.
	C.W. Lee	Biologist	LP NCI
	A. Hough	Medical Officer	Vanderbilt Univ.

COOPERATING UNITS (if any)

LEP, NIAMDD; Dept. of Path., Vanderbilt Univ.; and Dept. of Path., Health Sciences Center, State Univ. of New York, Stony Brook, N.Y.

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

SUMMARY OF WORK (200 words or less - underline keywords)

In the past two years the effort in this continuing study of connective tissues has been the analysis of the osteochondral junction and associated bone and cartilage and the joint cartilage in hemophilic arthropathy using the scanning electron microscope equipped with an energy dispersive x-ray analyzer. Now the effort is directed toward the chemical and morphologic changes in elastin during the development of "senile elastosis".

PHS-6040
(12-75)

Project Description:

Objectives: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular components.

Major Findings: 1. In collaboration with Drs. Hough and Sokoloff we have done an EDAX (energy dispersive analysis by x-rays) study of joint cartilage in hemophilic arthropathy using the scanning electron microscope as an electron probe. Abnormal inclusions containing iron associated with phosphorus are present within the chondrocytes in advanced hemophilic arthropathy.

2. Review paper for a Hayat Monograph was completed.

Significance: Demonstration of the potential of the scanning electron microscope, when used as an electron probe, to perform non-destructive elemental analysis on microscopic structures.

Proposed Course: Apply the scanning electron microscope with the energy dispersive x-ray analyzer to the analysis of connective tissues, particularly elastin and elastotic degeneration (senile elastosis).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00505-06 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pulmonary Carcinogens - their Relation to Morphological & Physiological Status of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.F. Stanton	Medical Officer	LP NCI
OTHER:	G. Akagi	Guest Worker	LP NCI
	A. Tegeris	M.D.	Pharmacopathics, Inc.
	O.T. Chorty	Ph.D.	U.S. Dept. of Agriculture
	C. Kommineri	D.V.M.	Environ. Protec. Agency
	E. Miller	Biologist	LP NCI
	M. May	Biologist	LP NCI

COOPERATING UNITS (if any) Johns-Manville Res. & Engineering Center, Manville, N.J.; Owens-Corning Fiberglass Corp., Toledo, Ohio; Roswell Park Mem. Center for Cancer Research; Pharmacopathics Inc., Richard B. Russell Agricult. Res. Center, USDA, Athens, Ga., Nat. Environ. Res. Center, EPA, North Carolina

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

This program is designed to investigate the major causes of pulmonary cancer in man through lung cancer induction in animals. We are currently studying the carcinogenic potential of subfractions of air pollutents and cigarette smoke condensate polynuclear aromatic hydrocarbons with our system of wax-retaining pulmonary implants in rats. The histogenesis of the neoplasms so induced is also being studied employing EM techniques, primarily.

Secondly, we are investigating the critical factors responsible for fiber carcinogenesis in the lung and pleura which depend on structural configuration of the particles of fibers such as the gypsums, metal whiskers, talcs, asbestoses, and fibrous glasses and a variety of other durable fibers. An effort to develop a workable hypothesis on the primary effect of the fiber on cells is in progress.

Project Description:

Objectives: 1. Investigations into pulmonary carcinogenesis with tobacco products and other potential pulmonary carcinogens for man.

2. Investigations into the factors affecting development of mesotheliomas experimentally induced with asbestos, fibrous glass and related fibrous minerals.

I. Tobacco Carcinogenesis: The final results of our initial tests with cigarette smoke condensate are at hand. They confirm our initial asumption that while the PAH fraction is by far the most carcinogenic component, other components, particularly the acidic fraction and aqueous methanol subfraction, augment carcinogenesis and are in themselves capable of pulmonary tumor induction. However, the fact that they augment pulmonary carcinogenesis would suggest that the removal of any of several components of either the acidic or neutral fractions of CSC might reduce carcinogenicity significantly. The results are also of significance in supporting the similar findings of skin painting experiments, thus reinforcing the idea that skin painting experiments are a valid means of determining pulmonary carcinogenesis.

Studies on the relationship of squamous metaplasia to the induction of neoplasia in the system has been carried out primarily by Dr. Akagi. Squamous metaplasia resulting from carcinogens would seem to have certain unique quantitative differences of significance.

Examination of other potential chemical pulmonary carcinogens is being carried out using the intrapulmonary pellet method. We hope to extend studies on tobacco smoke condensates to more specific fractions and to further examine other potential carcinogens such as extracted products of air-sample collected air-pollutants and specific chemicals such as benzene, carbon tetrachloride and the many specific carcinogenic hydrocarbons found in low levels in cigarette smoke and general atmospheric pollutants.

In the studies with intrapulmonary deposits of cigarette smoke condensates, a large series involving more than 4,000 rats has been initiated to determine the carcinogenic potential of 9 highly defined fractions of the PAH of cigarette smoke condensate isolated by the Agriculture Research Center, USDA, Athens, Georgia.

A large-scale contract, funded by DCCP has been undertaken to test the screening ability of the pulmonary pellet method to detect differences between cigarette smoke condensate from cigarettes with high and low nicotine content. In our own laboratory, we intend to test the method with several innovative cigarette smoke condensates and to explore the system with smoke condensates of other types that are common air pollutants. Briefly our plans call for testing nicotine and various fractions of cigarette smoke condensate and the condensate of cigarette smoke from cigarettes with altered or reduced tar and nicotine content as well as

extracts from specific air pollutants and other potential pulmonary carcinogens that may be lipid soluble.

II. Fiber Carcinogenesis: This year has been spent largely in the tedious job of counting and measuring individual fibers that have been used in our experiments with pleural implants of various fibers. Progress is slow, but accurate assessments of series of Al_2O_3 whiskers and Dawsonite crystals are beginning to show correlation with data currently in press on glass fibers.

With the virtual completion of analysis of the response to fiber distribution of different sized fibers of three major types namely, aluminum oxide, dawsonite and glass, interest can now be turned to more refined considerations of mechanism. Several factors are apparent. Irrespective of the type of fiber, carcinogenicity directly relates to increasing lengths of fibers that are at the limit of optical varibility or below. While it is easy to explain the lack of carcinogenicity in fibers sufficiently short to be completely entrapped by phagocytes, it is apparent that increasing length has an influence on carcinogenicity in fibers too long to relate to this phenomena. Several aspects of the situation seem involved in carcinogenicity. It is possible that carcinogenicity relates to surface contact with glass. Since finer fibers have far more surface area, one would expect a proportionately larger incidence of tumors with the finer fibers; however, it is unlikely that this could explain the complete lack of tumors in the controls implanted with large diametered glass. Another reasonable conjecture is that fiber ends are involved and that perhaps non-lethal penetrators of cell membranes is critical to carcinogenesis; however, if fiber ends were the critical factor one would expect proprotionally higher numbers of tumors from moderately long fibers than from extremely long fibers. Since this does not seem to be the case, the problem remains. Final data on these experiments should prove helpful in resolving these questions. However, the final resolution of this problem will probably depend on the results of experiments with fibers of precisely defined dimension which are in the process of being prepared from glass.

Regarding the screening of other materials, asbestos causes cancer not because it is asbestos, but because it contains durable fibers of exceptionally small diameter that are of exceptionally long length. The important corollary to this fact is that any durable fiber of comparable dimension may be as carcinogenic as asbestos. Our current efforts are being directed to searching and testing many types of fibers that are liable to contact with humans in one way or another. In particular, aerosols from dust-producing materials ranging from fertilizers to pollens as well as large diametered insulation materials are being examined. Furthermore, fibrous materials ingested orally are being studied. In general, testing consists of intrapleural application as previously described. However, we have recently begun a series of studies on the effect of intravenously administered fibers. This relates directly to the problem of orally ingested fibrous particles since absorption into the blood stream has been demonstrated in the rat.

Confirmation of the carcinogenicity of fibers for other species is also an item that will receive attention this year.

The effects of reduction in durability as a result of pyrolysis and the effect on surface properties by fiber coating will be investigated as time and space permit. Electron microscopy of candidate specimens of fibers and fine structure analysis of the early metaplastic alterations in pulmonary epithelium are in progress with the aim of understanding the preneoplastic changes that may be related to the induction of cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00516-11 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Comparative Studies on Neoplasms in Relation to Pathogenesis, Etiology and Diagnosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.F. Stanton	Medical Officer	LP NCI
OTHER:	G. Akagi	Guest Worker	LP NCI
	E. Miller	Biologist	LP NCI
	M. May	Biologist	LP NCI
	R.H. Adamson	Medical Officer	LCHPH NCI

COOPERATING UNITS (if any)

LCHPH, NCI
Pathological Technology Section, LP, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

Work centers on acquiring skills in the diagnosis and recognition of behavioral patterns in the tumors of laboratory animals employing techniques in light and electron microscopy and histochemistry. Major interest is currently focused on tumors of the Osborne-Mendel rat, since more than 4,000 aged females of this strain have been necropsied in this laboratory.

PHS-6040
(12-75)

Project Description:

Objectives: A study and accessioning of cancer in laboratory animals. Collection and development of competence in special areas of cancer morphology. Studies of histopathological and fine structure alterations related to the pathogenesis of disease induced by oncogenic viruses and other carcinogens.

Major Findings: An ongoing program has been the development of special competence in the histopathological and fine structural aspects of tumors of the lung, bone and mammary glands of rodents. Collection and examination of induced and naturally occurring bone tumors has been in progress for more than 5 years and will continue.

The collection and development of competence in lung tumors is essential for the work previously described and additionally serves as an aid to other investigators in the area and to the NCI Registry of Experimental Cancers. A current project on which Dr. Akagi and Ms. May serves is the study of the fine structure of the early squamous metaplasia preceding carcinoma at the site of implanted carcinogen pellets and the comparison of this change with the metaplasia associated with non-specific pulmonary inflammation which does not progress to cancer.

Interest in mammary neoplasia in rats is an offshoot of current long-term pulmonary experiments. Since over 50% of female strain OM rats develop mammary tumors in the second year of life it is necessary to surgically remove these in our experimental groups. As a result we have correlated data on incidence, growth rate and morphology in several hundred excised mammary neoplasms.

Proposed Course: We have through the past 5-6 years acquired an extensive catalog of neoplasms of all types that develop in aged female Osborne-Mendel rats. This data will be collated in an effort to establish normal incidence rates for our colony. In addition, time will be devoted to training in electron microscopy, studies of neoplasia in lower animals and preparation of a monograph on lung tumours in rats.

Publications:

1. A review of Pathology of Tumours in Laboratory Animals. Vol. 1: Tumours of the Rat, Part 1, V.S. Turusov, ed. (IARC Scientific Publications No. 5), Lyon, 1973, 212 pp. Environ. Res. 9: 94-98, 1975.

2. Stanton, M.F.: Tumors of bone. WHO Monograph Pathology of Tumours in Experimental Animals, vol. II, Tumours of the Mouse. In Press.

3. Stanton, M.F.: Tumors of the lung. WHO Monograph Pathology of Tumours in Experimental Animals, vol. I, pt. II, Tumours of the Rat. In Press.

Publications (Cont'd)

4. Dawe, C.J., Sonstegard, R., Stanton, M.F., Woronecki, D.E., and Reppert, R.T.: Intrahepatic biliary duct neoplasms in Catastomus commersoni. Symposium on Neoplasms in Aquatic Animals as Indicators of Environmental Carcinogens. Cork, Ireland, October 15-17, 1974. Vol. 21 "Tumors in Aquatic Animals" in the series Progress in Experimental Tumor Research; Homburger, F. (Ed.), S. Karger. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00521-06 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigations of Mechanisms of Tumor Induction by Polyoma Virus in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.J. Dawe	Medical Officer	LP NCI
OTHER:	W.G. Banfield	Medical Officer	LP NCI
	Y. Kurokawa	Visiting Fellow	LP NCI
	W.D. Morgan	Biologist	LP NCI
	J.E. Williams	Biologist	LP NCI
	C.W. Lee	Biologist	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

This project is an investigation of the significance and effects of interactions between epithelium and stroma during the transformation of epithelium from normal to neoplastic state. The systems currently being studied are: 1) Tumors of the submandibular salivary gland of mice, induced by polyoma virus; 2) Tumors of the forestomach and glandular stomach of mice, induced by a variety of chemical carcinogens. The general methods used involve neoplastic transformation of epithelium in the presence or absence of interaction with specific types of connective tissue (stroma). The results are evaluated on the basis of morphological and biological differences among the resulting neoplasms.

PHS-6040
(12-75)

Project Description:

Objectives: Most neoplasms, like normal tissues, show the ability of their stem cells to undergo some degree of differentiation and to manifest some amount of functional activity. Developmental biologists have shown that an important determinant in normal morphogenesis and differentiation of glandular organs is the interaction between the epithelial and mesenchymal components. Therefore it should follow that the nature of epithelial-mesenchymal interaction in progress when neoplastic transformation of epithelium takes place will in some degree affect the kind and the extent of differentiation of the resulting tumor. The object of this project is to test this hypothesis and to determine whether interference with epithelio-mesenchymal interactions can modify the effectiveness of carcinogenic agents.

Methods Employed: In previous years a number of experiments were carried out using the polyoma virus-mouse salivary gland tumor induction system. In the immediate past year, use of this system was continued, and development of a new system was started by Dr. Kurokawa, Visiting Scientist from Tohoku University, Sendai, Japan. Dr. Kurokawa's interest is in the influence of epithelio-mesenchymal interaction on the induction of stomach cancer by chemical carcinogens.

The work with these two systems will be reported separately.

A. Polyoma virus-mouse salivary gland system.

The use of Millipore filters as artificial but permeable barriers between epithelium and mesenchyme, or as a simple physical support for either type of tissue alone has been used in the following configurations:

1. Salivary epithelium opposite salivary mesenchyme - With and without polyoma virus.

2. Salivary epithelium opposite salivary epithelium - With and without virus.

3. Salivary mesenchyme opposite salivary mesenchyme - With and without virus.

4. Salivary epithelium alone, on one side of membrane - With and without virus.

5. Salivary mesenchyme alone, on one side of membrane - With and without virus.

6. Epidermal epithelium opposite dermal mesenchyme - With and without virus.

7. Epidermal epithelium alone on one side of membrane - With virus.

Cultures of these types were followed for periods up to a year, in specially modified culture chambers fitted with polyethylene tubes for exchange of nutrients and gases. At intervals, the Millipore membranes were removed and results evaluated by 1) light and electron microscopy of the cells on the membranes; 2) observations of the behavior of the recovered cells in Leighton tube cultures; 3) observation of the tumorigenic capacities of the cells after subcutaneous transfer to newborn mice.

Major Findings: Data in last year's Annual Report have been published and findings from extension of the work are now becoming available. Tumors developing from salivary epithelium transformed on Millipore membranes opposite mesenchyme were found to have the following characteristics as compared with tumors induced in vivo or in organ culture:

Trans-filter transformation	Transformation in vivo or in organ culture
1. Pleomorphic, highly aneuploid, poorly differentiated	1. Mixed epithelial and mesenchymal appearance, but only slightly aneuploid
2. Highly invasive	2. Non-invasive, well-encapsulated
3. Often containing areas of poorly-differentiated squamous cell carcinoma	3. No squamous cell component
4. In one instance, osteogenic as well as epithelial components present	4. No osteogenic component

These comparisons indicate great differences between tumors induced in salivary epithelium grown on Millipore membranes opposite mesenchyme, as compared with tumors induced in vivo, where normal epithelial-mesenchymal interaction occurs.

To determine whether the mesenchyme exerted any influence at all in the transfilter experiments, cultures of epithelium alone, transformed by PV on membranes, are under study. Several continuous culture lines of such transformed epithelium have been developed, but these have not yet been transferred to mice for tumor characterization, except in one instance, in which the resulting neoplasms were highly cellular, anaplastic spindle-cell tumors with no distinct epithelial features. Observations on additional examples are needed before this result can be interpreted.

Significance: From this work it is fully evident that neoplastic transformation of salivary epithelium under the culture conditions used results in tumors with properties distinctly different from those of tumors induced in vivo. This might be interpreted to mean that these differences were the result of interference with normal epithelial-mesenchymal interaction. Such a conclusion is not valid, however, since interference with epithelial-mesenchymal interaction was not the only variable that was different in vitro as compared with the conditions in vivo. In any culture system, nutritional, respiratory, neural, endocrine, and immunological environments differ from the corresponding ones in vivo. What our experiments have shown is that in transformation of salivary epithelium by polyoma virus, the influence of the virus may be greatly overshadowed by the influences of the microenvironment. While the virus seems to be essential for release of neoplastic expression, the pattern of that expression appears to be highly variable, depending on prevailing environmental factors.

Proposed Course: The experiments will be completed largely as outlined. Some additional procedures will be added. An additional control is needed, consisting of a PV-induced salivary tumor induced in vivo, then grown on Millipore membrane for variable periods, in the presence of PV. The properties of tumors derived from such a cell line will then be compared with those of tumors resulting from epithelial cells transformed in vitro on Millipore membrane. This will allow us to determine whether the culture environment can "guide" transformed cells to a common endpoint, regardless of whether transformation takes place in the presence of the culture conditions, or before the culture conditions are imposed.

B. Dr. Yuji Kurokawa's project: A study of epithelial-mesenchymal interaction during chemical carcinogenesis in glandular and squamous portions of the stomach.

Methods Employed: These experiments will be concerned with observations on tissues undergoing neoplastic transformation in vivo, although some of the manipulative procedures and exposure of tissues to carcinogens must be done in vitro.

The general experimental plan is to remove stomachs from embryo C3H/Bi mice at 15 days of gestation, and to transpose the mesenchymes of the glandular and squamous portions of the stomachs prior to exposing the recombinations to chemical carcinogens. The carcinogen-treated recombinations are then transplanted to syngeneic mice, and when tumors develop in these transplants, they are compared histologically and biologically with tumors induced in controls. Controls consist of (a) intact glandular and squamous portions of the 15-day embryonic stomach, treated with the same carcinogen and transplanted to syngeneic mice; (b) recombinations of glandular stomach epithelium with glandular stomach mesenchyme, and of squamous stomach epithelium with squamous

stomach mesenchyme, treated with the same carcinogen and transplanted to syngeneic mice. In addition, some experiments may be done in which the mesenchyme alone will be exposed to carcinogen. After a time sufficient to allow metabolism or degradation of the carcinogen, mesenchyme will be recombined with epithelium and observed in transplant for appearance of tumors.

By these procedures it should be possible to determine whether "phenotypic hybridization" of epithelial and mesenchymal components of an organ can alter the ability of the epithelium to respond to a given carcinogen, and the type of response the epithelium makes in such a changed stromal environment. For example, it might be postulated that a combination of glandular mesenchyme with squamous (forestomach) epithelium would give rise to adenocarcinoma rather than to squamous carcinoma. Such a postulate can be tested by these experiments, although it should be noted that we would not expect this particular postulate to prove correct.

Dr. Kurokawa has worked out the techniques for excision of stomachs from 15-day embryo mice, for recognizing and separating the glandular and squamous portions, and for separating the mesenchyme and epithelium of each portion and recombining as desired in short-term tissue cultures. He has also learned the methods for transplanting the recombinations into either newborn or adult mice, and for finding these transplants several weeks later. In other words, he has acquired all the techniques needed to execute the experimental plan.

Major Findings: The experimental and control transplants are still being set up after which an observation period of at least 1 year will be needed before results can be evaluated. It is expected that a full year (extending up to mid-October, 1976) will be required to complete setting up the experiment.

The preliminary work has progressed to the point where recombinations have been recovered from recipients, in the form of small cysts up to 0.5 cm. in diameter. Thus far the type of lining in these cysts has corresponded to the type of epithelium put into the recombination, rather than to the type of mesenchyme.

Since the results are not yet in, their significance cannot be discussed.

Proposed Course: It was originally planned to apply several chemical carcinogens to the experimental design outlined above. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is the only carcinogen that has been used as yet, but Dr. Kurokawa would like to apply ENNG (N-ethyl-N'-nitro-N-nitrosoguanidine, dimethylnitrosamine, diethylnitrosamine, dibutylnitrosamine, and N-methylbenzylnitrosamine to the same design. It seems essential to try several carcinogens, such as these, that are known to be carcinogenic

for esophagus or forestomach, in order to be reasonably certain that some tumors will be obtained.

Since Dr. Kurokawa's Fogarty Fellowship has a duration of only 1 year, it will be essential to obtain an extension of his Fellowship to permit completion of the work.

Publications:

Project A

1. Dawe, C.J., Morgan, W.D., Williams, J.E., and Summerour, J.P.: Inductive epithelio-mesenchymal interaction: Is it involved in the development of epithelial neoplasms? Proc. of the 2nd International Conference on Differentiation, Elsevier Press, Amsterdam, 1976, in press.

Project B

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00538-11 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

A Study of Phylogenetic Aspects of Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.J. Dawe	Medical Officer	LP NCI
OTHER:	W.G. Banfield	Medical Officer	LP NCI
	D. Small	Medical Officer	LMI NIDR

COOPERATING UNITS (if any)

LMI NIDR

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

SUMMARY OF WORK (200 words or less - underline keywords)

This is a continuing study in comparative oncology, designed to identify and characterize neoplasms in lower animals including all the cold-blooded vertebrates and all the invertebrates. By studying neoplasms in animals phylogenetically more remotely removed from man, it is hoped to gain a more complete understanding of the basic biology of neoplasia at all phylogenetic levels. It is expected that such studies will reveal properties of neoplasia not previously suspected to exist because of the excessively narrow scope of studies limited to the higher animals and man. Important differences in the immunologic, biochemical, and regenerative characteristics of the lower animals should serve to emphasize the relevance of these characteristics to the development of and the susceptibility to neoplasia. In addition, by study of neoplasms in lower animals under feral conditions, these neoplasms may be found to serve as indicators of environmental tumorigenic agents. Neoplasms are collected, either in the field or through submittal from outside sources, and are studied morphologically and biologically. Light and electron microscopy are used with fixed material. Tissue culture, transplantation, and induction methods are applied to study phenomena such as regenerating dedifferentiation, autotomy, graft rejection, tumor progression, and others.

750

Project Description:

Objectives: The continuing major objective of this project is to perform comparative studies of neoplasia in invertebrate and lower vertebrate animals. Such studies serve two main needs: 1) To gain a comprehensive view of the biology of neoplasia throughout the animal phyla and thus to achieve a more detached perspective of the problem of cancer in man; 2) To find and make use of neoplastic diseases in lower animals in 2 ways: a) by adapting them to experimental uses in studies of the properties and mechanisms of genesis of cancer; b) by using them as indicators of environmental tumorigenic agents possibly related to the epidemiology of human neoplasia.

Methods Employed: Neoplasms are collected, either in the field or through submittal from outside sources, and are studied morphologically and biologically. Light and electron microscopy are used with fixed material. Tissue culture, transplantation, and induction methods are applied to study phenomena such as regeneration, dedifferentiation, autotomy, graft rejection, tumor progression, and others.

Major Findings: A. Much of the reporting investigator's work in this project has been concerned with organizational, advisory, and educational activities. Among these were:

1. Completion of the editorial duties in follow-through of the symposium on Aquatic Tumors as Indicators of Environmental Carcinogens, sponsored by the International Union Against Cancer. This meeting was held at University College, Cork, Ireland, October 16-18, 1974. The 25 papers, including 2 by the reporting investigator, have now been edited and are in page proof for publication in vol. 20, entitled Tumors in Aquatic Animals, of Progress in Experimental Tumor Research, S. Karger, Basel. The reporting investigator, with Drs. S.R. Wellings and D. Scarpelli is a guest editor of this publication.

2. Participation in the organization and conduct of a Workshop on Malignant Lymphomas in Esocidae, held on October 9-11, 1975 at the State Veterinary Institute of Sweden in Stockholm, Sweden under sponsorship of the International Union Against Cancer and the Swedish Cancer Society. At this workshop the reporting investigator was Chairman of a scientific session; with Drs. W.G. Banfield and R.A. Sonstegard presented a paper (in press in J. Natl. Cancer Inst.; see Publications); and was assigned by the participants to prepare a summary report of the proceedings for submittal to the Journal of the National Cancer Institute. The first draft of this report has been completed and sent out to all other participants for final revisions prior to submittal to the Journal.

3. With Dr. H. Kraybill (DCCP), J. Harshbarger (Smithsonian), and R. Tardiff (EPA), co-organizer of a meeting on Aquatic Pollutents and Biological Effects with Emphasis on Neoplasia, to be sponsored by the New York Academy of Sciences with support from NCI, EPA, ERDA, and NIEHS,

on September 27-29 at the Barbizon Hotel, N.Y.C. This meeting will bring together two categories of workers that have not previously met in the same forum: a) those who have been studying neoplasms in aquatic animals, and b) those who have been studying chemical pollution of water. The proceedings are to be published in the Annals of the N.Y. Academy.

4. Participated in a meeting at Eastern Virginia University Medical School, convened to determine whether a society and a journal of Comparative Pathology should be organized. Further steps have subsequently been taken to test the feasibility of starting such an organization.

5. With Drs. D. Scarpelli, A. Rosenfield, and J. Harshbarger, was a member of organizing and program committee for the Workshop on Pathology of Molluscs, held September 7-9, 1975, at the Wye Institute, Talbot County, Maryland. Participated in this meeting as Chairman of a session on Neoplasms in Molluscs. Proceedings of the meeting are in press as a volume of Reports of the U.S. Department of Fisheries.

6. During the past year, the Upper Chesapeake Bay Survey, for which the reporting investigator served as a consultant, was completed by the Department of Natural Resources of the State of Maryland. Activities in this work were therefore concluded.

B. Investigative Work.

1. Studies concerned with case reports and tissue culture of a chondrosarcoma in a corn snake, and of a nephroblastoma in a rainbow trout, as described in last year's report are being continued. It is hoped to complete reports for publication on these two subjects in the coming year. Continuous cell culture lines of both these tumors have been developed.

2. With Dr. W.G. Banfield, R.A. Sonstegard et al (see publications), initiated and completed a morphological study of lymphoma cells of epizootic malignant lymphoma of northern pike. Two types of intracytoplasmic structures were found that had not been previously reported in these tumors. One, which we have called cylindroid lamella-particle complex, is identical to structures found in human leukemias and lymphomas. Their functional nature is not known. Two possibilities that have received the most consideration are: 1) that the cylindroid bodies are an unusual form of manifestation of viral infection and replication; 2) that they are an indication of some anomaly of protein synthesis.

A second intracytoplasmic structure discovered in the pike lymphoma has been called nucleoid body, because of staining properties similar to nuclear chromatin. These have not been characterized as yet by EM, and their functional nature is unknown.

3. In collaboration with workers at the Nanaimo Laboratory of Fisheries Biology at Nanaimo, British Columbia, and Mr. Michael Poore of the Section of Histopathological Technique, Laboratory of Pathology, a study of tumors of the parabranchial body in Pacific codfish has been undertaken. These tumors are common in this species, and are usually bilateral. It has been claimed by a Norwegian investigator that similar tumors in Atlantic cod contain argyrophil/argentaffin granules and are comparable to carcinoid tumors in man.

Our study shows: 1) that there is little if any resemblance between the parabranchial tumors and carcinoid tumors; 2) that the normal parabranchial body epithelium contains neither argyrophil nor argentaffin cells; that the "tumor" cells of the parabranchial body masses do contain argyrophil, but not argentaffin granules; that electron-microscopically there is little resemblance between the "tumor" cells and normal parabranchial body epithelium.

We now strongly suspect that the parabranchial body "tumors" of cod are not neoplasms at all, but infestations by a protozoan parasite.

It is planned to press this work to the point where we are secure on the question of neoplasia vs. parasitic disease, and then to publish the results of the light and electron microscopic study. If a protozoan parasite can be identified as the cause of the parabranchial body "tumors", we will have identified another example, comparable to protothecosis, in which a parasitic organism masquerades as neoplastic cells.

Publications:

1. Dawe, C.J., Banfield, W.G., Sonstegard, R., Lee, C.W., and Michelitch, H.J.: Cylindroid lamella-particle complexes and nucleoid intracytoplasmic bodies in lymphoma cells of northern pike (Esox lucius). Vol. 20 "Tumors in Aquatic Animals" in the series Progress in Experimental Tumor Research; Homburger, F., (Ed.), S. Karger, in press.

2. Dawe, C.J., Sonstegard, R., Stanton, M.F., Woronecki, D.E., and Reppert, R.T.: Intrahepatic biliary duct neoplasms in Catastomus commersoni. Vol. 20 "Tumors in Aquatic Animals" in the series Progress in Experimental Tumor Research; Homburger, F., (Ed.), S. Karger, in press.

3. Banfield, W.G., Dawe, C.J., Lee, C.W., and Sonstegard, R.: Cylindroid lamella-particle complexes in lymphoma cells of northern pike (Esox lucius). J. Natl. Cancer Inst., in press.

4. Dawe, C.J.: Neoplasms of fish. In chapter entitled Tumors, by R.A. Squire, D.G. Goodman, T. Frederickson, M.H. Levitt, C.H. Lingeman, M. Valerio, J.D. Strandberg, J.C. Harshbarger, and C.J. Dawe, in book entitled: Pathology of Laboratory Animals, ed. by K. Benirschke, F.M. Garner, and T.C. Jones; Springer-Verlag, New York.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00539-02 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Lysis and Phagocytosis of Antibody-Sensitized Chicken Red Cells by P388D_1 Mouse Macrophage Tumor Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.S. Koren	Medical Officer	I NCI
OTHER:	W.D. Morgan	Biologist	LP NCI
	C.J. Dawe	Medical Officer	LP NCI

COOPERATING UNITS (if any)

I NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been completed. The project resulted in the production of a 4 minute black and white, 16 mm. optical sound film that clearly illustrates an example of antibody dependent cell mediated cytotoxicity. In the film P388D_1 mouse macrophage tumor cells (effectors) lyse and phagocytize chicken red cells (targets) that had been modified with 2,4,6-trinitrophenyl (TNP) and coated with mouse anti-TNP antibodies.

The Tissue Culture Association Film Collection Committee requested a copy of the film. Their request was granted and the film is available from: W. Alton Jones Cell Science Center, Film Library, Lake Placid New York 12946.

PHS-6040
(12-75)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00540-01 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Contractile Tissues in Organ Cultures of Mouse Submandibular Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.D. Morgan	Biologist	LP NCI
OTHER:	C.J. Dawe	Medical Officer	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

Time-lapse photographs had shown synchronous and asynchronous contractions in organ cultures of 14 day embryo mouse submandibular glands. These photographs showed that contractions began about the sixth day of culture and continued for more than 30 days. The contractions appeared mixed: some synchronous and occurred frequently; others appeared independent and occurred infrequently. A documentary film of contractions such as these in organ cultures of mouse submandibular glands has not been produced - to our knowledge. Therefore, additional photographic studies will be made of these glands in organ culture in an effort to determine precisely the element(s) of contraction. Contractile elements and parasympathetic nerve elements will be studied histochemically and electron microscopically. Results will be described in a written report and presented in a documentary film.

PHS-6040
(12-75)

Project Description:

Objectives: The objectives of this project will be 1) to produce a documentary film that clearly shows contractions in organ cultures of mouse embryo submandibular glands; 2) to determine whether the contractile element(s) observed in mouse submandibular glands appear to be myoepithelium or smooth muscle; and 3) to determine whether parasympathetic nerve elements in these glands affect contractions observed there.

Methods Employed: Organ cultures of submandibular glands of 14 day embryo mice will be cultured on glass in modified Rose tissue culture chambers and in tube cultures. Chamber cultures will be photographed, using techniques of time-lapse photography. Tube cultures will be studied by histochemical and electron microscopic techniques. Glands with and without their submandibular ganglia will be studied. Time-lapse photography will be used to make a permanent record of contractions; while histochemistry and electron microscopy will be used to identify components of myoepithelium, smooth muscle, and parasympathetic nerve.

Major Findings: Contractions in organ cultures of 14 day embryo mouse submandibular glands began approximately 6 days after start of culture and continued for more than 30 days. Time-lapse photography show the contractions to be mixed: some appeared synchronous and occurred frequently, and others appeared asynchronous and occurred infrequently.

Significance: Contractile cells such as myoepithelium play a significant roll in some tumors of salivary glands (pleomorphic adenomas or so-called mixed tumors, for example). A documentary film on the action of these cells in normal glands may increase our understanding of the role played by contractile cells in neoplasms of salivary glands.

Proposed Course: The results obtained from this project will be described and illustrated in a written report and further illustrated in a documentary film.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00541-01 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Phagocytosis of Yeast by White Blood Cells (Film)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.J. Chusid	Medical Officer	LCI NIAID
OTHER:	W.D. Morgan	Biologist	LP NCI

COOPERATING UNITS (if any)

LCI NIAID

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been completed. During the year a 4 minute color film was produced. The film illustrates the movement of polymorphonuclear leucocytes in vitro and several instances of phagocytosis of killed yeast cells (Candida albicans) by leucocytes.

In collaboration with the Laboratory of Clinical Investigation, NIAID, and NIH Information, I contributed excerpts from this film to help produce a teaching film for junior high school students. The teaching film is entitled "Invaders and Defenders" and is distributed by Association Sterling, 1701 N. Fort Meyer Drive, Arlington, Virginia 22209.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00560-10 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Electron Probe and Scanning Electron Microscope

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	S.B. Doty	Medical Officer	LBS NIDR
	E.G. Trams	Medical Officer	LNC NINCDS
	C.J. Lauter	Medical Officer	LNC NINCDS
	C.W. Lee	Biologist	LP NCI

COOPERATING UNITS (if any)

LNC, NINCDS; LBS, NIDR

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this work is to develop the instrumentation and techniques so that the x-ray and other analytical capabilities of the scanning electron microscope can be applied effectively to biological problems. The subjects of present interest are elastin and stratified squamous epithelium.

PHS-6040
(12-75)

Project Description:

Objectives: Application of electron probe and scanning electron microscope to the analysis of biological specimens.

Major Findings: A stage for direct light microscopic observation of tissue sections and cathode luminescence in the scanning electron microscope is in use allowing direct correlation of light microscopic and SEM structures.

Significance: The light microscope for the scanning electron microscope will allow for light optical microscopic visual centering of the object of interest under the electron beam for correlation with the secondary electron and specimen current images and for direct visualization of cathode luminescence.

Proposed Course: 1. Apply the scanning electron microscope with energy dispersive x-ray analytical attachment (EDAX) to the analysis of connective tissues, particularly elastin, and to the analysis of stratified squamous epithelium, squamous carcinomas and basal cell tumors with especial emphasis on sulfur.

2. Use the light microscope stage constructed for the scanning electron microscope in a study of the cathode luminescence of elastic fibers.

3. Construct or modify the existing light microscope stage for the ETEC scanning electron microscope to be used for energy dispersive x-ray analysis.

4. Apply EDAX analysis to collaborative efforts as they arise.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00561-06 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Collaborative Research and Other Activities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M.F. Stanton Medical Officer LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Twenty percent of the normal work week is spent in the following activities: 1. Member, Smoking and Health Review Board-OD; 2. Member, Asbestos Protocol Subcommittee of the HEW Committee on Coordinated Toxicological Research and Related Programs; 3. Project Officer, NCI, DCBD-NO1-CB-33887, Small Animal Holding Facility; 4. Project Officer, NCI, DCBD-NO1-CB-43912, Hematoxylin Substitute Development; 5. Co-Project Officer, NCI, DCBD-NO1-CB-33874, Registry, Tumors in Lower Animals; 6. Co-Project Officer, NCI, DCCP-NO1-CP-3374, Standardized Lung Pellet Technique; 7. Member, Editorial Board Monograph Series, IARC-WHO, Lyon, France.

These activities primarily involve investigations into the carcinogenicity of asbestos, other durable fibers, and tobacco products. Additionally they involve the collection of data on neoplasms in laboratory and histochemical investigations as an aid to diagnosis.

PHS-6040
(12-75)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00852-23 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Chu	Medical Officer	LP NCI
OTHER:	P.R. DelVecchio	Super. Res. Biologist	LP NCI
	S.H. DeWitt	Res. Biologist (Cytol.)	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

SUMMARY OF WORK (200 words or less - underline keywords)

Provides complete diagnostic service in exfoliative cytology, medical cytogenetics, and fine needle aspiration cytology. In addition, collaborates in various clinical research projects utilizing special techniques including special staining and tissue culture techniques.

PHS-6040
(12-75)

Project Description:

Objectives: 1. To provide the staff of the Clinical Center with an accurate and complete exfoliative cytology service as well as diagnostic cytogenetics (karyotyping).

2. To collaborate in various clinical research projects evaluating cancer therapy, the hormonal status of the cancer patients, the course and natural history of the cancer lesions and the anatomical and physiological changes in the human body associated with various pathologic conditions.

3. Development of better diagnostic techniques.

The specific objectives include:

A) Cytology

1. Continued improvement in cytologic techniques in establishing definitive diagnosis.
2. Improvement in fine needle aspiration cytologic technique.
3. Establishing cytologic characteristics of cell types seen only in fine needle aspirations.
4. Setting up special stain techniques which may be used for general cytology or special research problems.

B) Diagnostic Cytogenetics

1. Improvement in short term culture system to assure of successful harvesting of in-vitro cells.
2. Improvement in staining techniques.
3. Improvement on special techniques such as Giemsa bandings of chromosomes and sister chromatid exchange methods.

Methods Employed:

1. Special procedures in addition to the standard Papanicolaou stain are:

A) Millipore filtration of all fluids
B) Special stains:

1. Feulgan Reaction for sex chromatin bodies
2. Acid fast, Pneumocystis, Methenamine silver, Brown Brenn for various organisms and fungi
3. PAS, Wright Giemsa, iron stain, melanin stain, congo-red, oil-red-O, mucicarmine for various specific reactions

2. Short term cultures of peripheral blood, bone marrow cells and tumor cells found in body fluids are the standard diagnostic methods.

In addition, special techniques involving trypsin digestion followed by G-Banding (Giemsa bands) are routinely offered.

Biopsy materials of skin, gonads, tumors are cultivated by tissue culture technique and cells harvested for chromosomal analysis.

Patient Material: The figures compiled below are for the last 12 months.

March 1975 - February 1976

Type of Material Examined:	Accessions	Smears
Vaginal and Cervical	1,332	2,273
Other (Ascitic, pleural, gastric, prostatic, urine, bronchial washings, sputa, spinal fluids, sex chromatin, wound washings, breast, oral, aspirations, chromosome analysis and special procedures)	2,772	6,753
Total	4,104	9,026

Institutes	Accessions	Smears
NCI	2,067	4,727
NHLI	376	858
NIAID	249	717
NIAMDD	538	880
NIMH	64	142
NICHHD	309	758
NEI	2	3
NINCDS	271	428
CC (EHS)	17	35
NIDR	182	364
Referred	29	114
Total	4,104	9,026

Major Findings: Major contributions of the Cytopathology Section:

1. Providing prompt services and early diagnosis in cytological materials.
2. Providing cytologic evaluation and estimated ranges of either relapse or remission in the continual management of meningeal leukemia.
3. Providing cytological evaluation of therapeutic effects on urinary specimens.
4. Providing chromosomal analysis for clinical studies as well as in the establishment of definitive malignancies.
5. Providing cytologic methods to research projects specifically, fine needle aspiration in thyroid nodules and bone lesions.

Significance: 1. The diagnostic value of exfoliative cytology in the clinical management of various disease states is an established fact.

2. Aspiration cytologic techniques are useful in establishing diagnosis on metastatic diseases and/or recurrent malignancies. In such instances, the fine needle aspiration technique eliminates the more involved incisional biopsy and therefore is more economical to the clinicians as well as to the patients.

3. Chromosomal analysis is a way of establishing definitive diagnoses in selected patients with endocrine disorders, congenital defects and also in some malignancies.

Proposed Course: 1. Continue to provide a complete cytodiagnostic service for the entire staff of the Clinical Center in various research projects.

2. Continual improvement and development of established as well as new techniques in cytologic and cytogenetic fields.

Publications:

1. Wood, T.A., Jr., DeWitt, S.H., Chu, E.W., Rabson, A.S., and Graykowski, E.A.: Anitschkow nuclear changes observed in oral smears. Acta Cytol. 19: 434-437, 1975.

2. Dellon, A.L., Chu, E.W., Hall, C.A., and Chretien, P.B.: Cytology of bronchial washings. A study of patients with malignant neoplasms of the head and neck. Arch. Otolaryngol. 101: 465-466, 1975.

3. Didier, M.L., Ablashi, D.V., Oie, H.K., Armstrong, G.R., Chu, E.W., and Rabson, A.S.: Some biological properties of Herpesvirus saimiri (HSV) from chronically infected monolayer and suspension cultures. Monograph (IARC).

4. Gershengorn, M., Pocaven, M.A., McClung, M.R., Broadus, A.E., Chu, E.W., and Robins, J.: Elevated histaminase activity in needle aspiration biopsy of the thyroid for diagnosis of medullary carcinoma. N. Engl. J. Med.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00517-35 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathological Technology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.J. Coolidge	Supv. Bio. Lab. Tech. (Micro)	LP NCI
OTHER:	L.B. Thomas	Medical Officer	LP NCI
	C.J. Dawe	Medical Officer	LP NCI

COOPERATING UNITS (if any)

FCRC - NCI Project 14

LAB/BRANCH

Laboratory of Pathology

SECTION

Pathological Technology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	0	9

SUMMARY OF WORK (200 words or less - underline keywords)

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

July 1, 1975 - June 30, 1976

Number of investigators	87
Number of pieces of tissue	51,167
Number of bottles of tissue	10,787
Number of blocks cut	34,000
Number of blocks cut serially	968
Number of frozen blocks cut	10
Number of blocks recut	1,106
Number of slides stained H & E	43,315
Number of slides stained special	3,578
Number of unstained slides	8,987

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00582-05 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chronic Mouse Cytomegalovirus Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	R.E. Levinson	Biologist	LP NCI
	R.D. Smith	Medical Officer	Univ. of Cincinnati

COOPERATING UNITS (if any)

University of Cincinnati School of Medicine

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the possible reactivation of chronic mouse cytomegalovirus infection by immunosuppression and anti-lymphocytic serum.

PHS-6040
(12-75)

Project Description:

Objectives: Study the mechanisms of chronic murine cytomegalovirus infection in the salivary glands and lymphoid tissue of mice chronically infected with this virus and effect of anti-lymphocyte serum on infection.

Methods Employed: Tissue culture, immunofluorescence, electron microscopy.

Significance: The mouse cytomegalovirus causes chronic infection in the submaxillary glands, lymph nodes, and spleens of many strains of mice. Infection in the lymph nodes and spleen probably occurs in lymphocytes. The murine cytomegalovirus belongs to the herpesvirus group of animal viruses, along with the Epstein-Barr virus and Herpesvirus saimiri.

Proposed Course: Part of this study is completed. Results indicate that chronic CMV infection does not enhance or accelerate natural development of lymphoma in mice. Preliminary results indicate that the cause of infection is drastically altered by anti-lymphocytic serum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00585-03 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Histological Evolution of In-Situ Carcinoma in the Vagina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	G.W. Geelhoed	Medical Officer	Dept. Surg., G.W. Univ.

COOPERATING UNITS (if any)

George Washington University School of Medicine

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to review biopsy material from the vagina of patients who have had a previous carcinoma of the cervix in order to evaluate the vaginal epithelium for early in-situ changes.

PHS-6040
(12-75)

Project Description:

Objectives: Clinical-pathological study of women with multicentric squamous cell cancers in the ectogenital epithelium and relationship of in-situ vaginal cancer to subsequent invasive cancer.

Major Findings: Histological studies made on women with sequential vaginal biopsies have demonstrated progression from severe epithelial atypia, through dysplasia, to carcinoma in-situ. Like carcinoma of the cervix, in-situ vaginal tumors have an invasive potential. In-situ vaginal tumors associated with carcinoma of the cervix seem to form a distinctive clinical syndrome.

Significance: Many women with cancer of the uterine cervix subsequently develop epithelial cancers in the vagina and/or vulva. These multiple cancers have been considered to arise in an epithelial region predisposed to malignancy. This region has a common cloacal origin embryologically.

Proposed Course: Project is still in effect. Patients will be added to the study as they are diagnosed. A report has been accepted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00591-02 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunofluorescence Studies on Human Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	R.E. Levinson	Biologist	LP NCI
	R.S. Adelstein	Medical Officer	IR CB NHLI

COOPERATING UNITS (if any)

IR CB NHLI

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range of this project to study the differences of the myosins and other proteins present in normal tissue, rhabdomyosarcomas, and other soft tissue tumors.

PHS-6040
(12-75)

Project Description:

Objectives: Use of immunofluorescence on frozen section material to look for novel or unusual proteins synthesized by human tumors.

Methods Employed: Immunofluorescence immunology.

Major Findings: Dedifferentiated rhabdomyosarcoma cells produce a new type of myosin immunologically and biochemically related to myosin present in fibroblasts. Myosin might be helpful in diagnosis of rhabdomyosarcomas.

Significance: The purpose is to search for novel proteins in tumors and to determine if these proteins can be useful in the early diagnosis of cancer, diagnosis of precancerous lesions, and for predicting prognosis.

Proposed Course: Test a variety of tumors by immunofluorescence using antibodies prepared against purified proteins that have been isolated from tumors, fetal tissue, normal organs, or from other sources.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00592-02 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

A Morphologic Investigation of Virus Induced Membrane Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.J. Triche	Medical Officer	LP NCI
OTHER:	J.C. Costa	Medical Officer	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

This project attempts to elucidate the relationship between membrane antigens and an Fc receptor which appear in Herpes simplex type II-infected cultured human retinoblastoma cells. Further, both of these will be related to the increased Con A lectin binding ability of HSV II-transformed cells previously noted in the literature. The appearance of an Fc receptor and increased lectin binding of a following infection time when viral-directed membrane antigens are appearing suggests a relationship between these three membrane events. We suspect the Fc receptor is a viral-directed membrane-associated protein, as are the increased Con A binding sites. We are attempting to prove or disprove this assumption using ferritin and hemocyanin-labelled antibodies and lectins. The ability to bind host IgG may confer survival advantages to HSV-infected cells, enhancing both viral replication and latency.

PHS-6040
(12-75)

Project Description:

Objectives: 1. To characterize the membrane antigen (MA) of herpes simplex with regard to the time of appearance following infection, the distribution, and the association with lectin receptor sites, in cultured retinoblastoma cells.

2. To compare these results with those in the literature, and to determine the association, if any, of these membrane sites with the increased agglutinability of transformed cells by the lectin concanavalin A.

3. To compare the MA sites with the Fc receptor which also appears following HSV infection.

Methods Employed: 1. Freeze etch electron microscopy, utilizing high resolution platinum-carbon replicas of fractured and etched cell surfaces examined in the transmission electron microscope.

2. Labelling of these surfaces with ferritin or hemocyanin-tagged lectin or immunoglobulin.

Significance: 1. The known increased agglutinability of transformed cells by plant lectins (such as concanavalin A) may be related to an alteration in number or distribution of cell surface receptors for lectins, which may coincide with the appearance of viral-associated material in the membrane.

2. The HSV-directed Fc receptor may play a role in virus latency and oncogenesis.

3. A double label study, labelling both viral antigen-associated membrane sites and lectin binding sites, should provide unequivocal evidence, pro or con, of the means whereby viruses induce increased cell agglutinability, a property of cells associated with oncogenesis.

Proposed Course: Considerable time will be spent preparing reagents for labelling the membranes as well as installing the freeze etch device. Once the technological aspects are assimilated, the project will progress forthrightly through preliminary experiments and finally through repetitive testing to establish reproducibility. Later, other parameters, such as cell type, virus type, temperature, type of lectin, etc. will be evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00533-03 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Oncogenic DNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.C. Costa	Medical Officer	LP NCI
OTHER:	A.S. Rabson	Medical Officer	DCBD NCI
	T.J. Triche	Medical Officer	LP NCI
	G.R. Mendoza	Medical Officer	A&R NIAMDD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology & Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

The general scope of this project is to study biological properties of human herpesviruses and human papovaviruses tentatively associated with tumors in human patients. At present the following topics are under investigation: 1) Characterization of the Fc binding site on HSV infected cells and its possible biological role. 2) Characterization of a new human papovavirus isolate closely related to BK virus in terms of oncogenicity in vivo and ability to transform cells in vitro.

Project Description:

Studies are in progress to elucidate the significance of a binding site for the Fc fragment of immunoglobulin appearing on herpes simplex infected cells. Experiments done suggest that the appearance of the Fc binding site requires the synthesis of early products (pre-viral DNA synthesis) and that is probably a subset of viral coded antigens.

It has been confirmed that engagement of the binding site with rabbit IgG and with Fc fragments of IgG interferes with viral production. It is hoped that this phenomenon will represent a new approach to the understanding of herpesvirus latency.

The oncogenic potential of MM virus, a human papovavirus isolate, has been tested. Experiments in hamsters have shown that the virus, at the doses used, is oncogenic when injected intracranially but not when injected subcutaneously or intravenously. Intracranial injection caused ependymomas in the hamster. MMV is able to transform cells in vitro and three lines (2 hamster, and 1 mouse line) have been established in our laboratory. Because one cell line was derived from NIH nu/nu mice, the antibody response to papova tumor antigen has been tested in the immunodeficient host. It has been found that in the nu/nu mouse no antibody response is elicited by the T antigen present in the tumor cells inoculated into the animals.

With reagents developed in our laboratory human lines derived from CNS tumors have been tested for the presence of papova-T antigen. None of the cell lines examined had antigen. 100 sera from cancer patients have been examined for the presence of T antibody. No reactivity was found in the sera.

Proposed Course:

Experiments are in progress to determine if the Fc binding site appears in neurons and to see if latency established in vivo in the mouse model can be maintained in vitro by engaging the Fc binding sites that may be present in the latently infected cells.

The topology of the Fc binding sites is being determined by the use of freeze-etching techniques.

Cell cultures derived from human osteosarcomas are being examined for the presence of antigens reacting with papovavirus T antibodies.

Publications:

1. Costa, J., Yee, C., Tralka, T.S., and Rabson, A.S.: Hamster ependymomas produced by intracerebral inoculation of a human papovavirus. J. Natl. Cancer Inst. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00534-02 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure and Assembly of Virulent and Vaccine Polioviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N.A. Young	Medical Officer	LP NCI
OTHER:	M.E. Proctor	Postdoctoral Fellow	LP NCI

COOPERATING UNITS (if any)

James Nakano, Enterovirus Unit, Center for Disease Control
Atlanta, Georgia

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

The structural lability in SDS gels of the largest virion polypeptide (VP1) of type 1 poliovaccine has been successfully exploited as a marker to distinguish neurovirulent from attenuated strains of poliovirus. Similar lesions were not identified in the polypeptides of types 2 and 3 poliovirus vaccine strains.

The antigenic characteristics of isolated polypeptides of these viruses are currently under investigation by the technique of radioimmunoprecipitation. Proteins purified by SDS gel electrophoresis have proved to be poor immunogens, thus hindering our attempts to compare these polypeptides directly.

Project Description:

We have employed standard virologic and immunologic techniques to investigate molecular correlates of diminished neurovirulence in Sabin vaccine strains of poliovirus by comparing the structure, assembly, and immunologic reactivity of their polypeptides. The most important finding has been that the structural lability of VP1 of type 1 vaccine strains can be used as a marker to differentiate them from neurovirulent strains. Six strains received as coded unknowns from the CDC were correctly identified as of vaccine or wild origin. Further studies are designed to elucidate whether "leakiness" (backmutation to properties associated with virulence) can be detected in type 1 polioviruses isolated serially from the stools of children who have been fed vaccine. We have also examined the polypeptides of type 2 and type 3 polioviruses by SDS gel electrophoresis, but similar molecular lesions were not detected. The significance of these findings is that protein analysis may have potential in determining whether human paralytic poliomyelitis following vaccine administration is caused by the vaccine virus or by concomitant infection with naturally-circulating neurovirulent strains.

Future investigations will focus on the antigenic specificity of the 3 major virion proteins of polioviruses. These proteins have been separated preparatively on polyacrylamide gels and used to immunize rabbits. Comparisons of these proteins from different virus strains will be made by the technique of radio-iodination and immunoprecipitation. To date our efforts have been hampered by the poor immunogenicity of the isolated polypeptides when injected into rabbits. Further attempts will be made to produce antisera with adequate precipitating antibody titers by using repeated booster injections and various adjuvants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 00535-01 LP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biologic and Virologic Characterization of Breast Cancer Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N.A. Young	Medical Officer	LP NCI
OTHER:	J.A. Strauchen	Clinical Associate	LP NCI

COOPERATING UNITS (if any)

Marc Lippman, M.D., Medicine Branch, NCI
Pradman Qasba, Ph.D., Lab. of Pathophysiology, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

Three lines of breast cancer cells have been established in continuous culture. These cells show evidence of differentiated function manifested by the synthesis of alpha lactalbumin and casein and growth responsiveness to estrogen and insulin. Oncornaviruses have not thus far been detected in the cells by a variety of techniques, including electron microscopy and assays for 70S RNA and reverse transcriptase.

Project Description:

Studies of the possible role of oncornaviruses in the etiology of human breast cancer have been hindered by the lack of well-characterized pure populations of breast cancer cells. We have processed cells from malignant effusions and have succeeded in developing 3 continuous lines. Investigations of these cells have developed in 2 major directions: 1) biologic characterization of the cells and 2) a search for evidence of oncornavirus expression. Major findings to date on one of these cell lines which has been the most thoroughly studied include stable karyotypic abnormalities; ultrastructural features compatible with secretory epithelial cells; synthesis of differentiated proteins such as alpha lactalbumin and casein; presence of receptors for estrogen and other steroid hormones; growth responsiveness to estrogen and insulin. No complete (infectious) oncornaviruses have been detected in these cells as assayed by the presence of 70S RNA in particles banding at p 1.16 - 1.18; reverse transcriptase activity; electron microscopy; co-cultivation with a variety of human and animal indicator cells.

In the coming year, the major effort will be directed toward 1) characterization of the other breast cancer cell lines by similar techniques; 2) a search for oncornavirus gene expression, in the absence of complete infectious virus, as measured by molecular hybridization and radioimmunoassays for nucleic acid sequences and proteins related to mouse mammary tumor virus and the Mason-Pfizer monkey virus.

Annual Report of the Image Processing Unit,
Diagnosis Branch, Division of Cancer Biology and Diagnosis
National Cancer Institute

The Image Processing Unit constructs for and conducts research in the reproducible characterization of cell morphology and kinetics, employing living as well as fixed and/or stained material as subjects for interactive biologist examination and measurement. To this end, the major attention during the year was devoted to the construction of software, optical and digital computer support for the real time role of the biologist in the characterization and measuring role. In view of the generality of many of the image analysis/processing algorithms, and the device independence of much of the hardware, a very large proportion of the work of the unit bears directly on such diverse fields as electron microscopy, ultrasonics, nuclear medicine and radiology (especially computerized axial tomography).

Presently, the senior staff of the unit consists of the Unit Head and four senior investigators (two of the latter hold "expert" appointments). We expect a sixth senior investigator in the person of a Fogarty International Fellow to join our group prior to the end of the reporting period.

Following are the highlights of the research in the Image Processing Unit:

Automatic Cell Analysis (Project No. Z01 CBD 08476-04 IMP):

A. Tissue Culture: Extensive culture and time lapse cinematographic studies of macrophage line P388D (provided us by Wunderlich and Koren) were carried out during the year. Since we intend to use this line of macrophages for our first set of experiments with computer-controlled microscopic observation of living material, we have devoted much time to cell cycle time and related studies.

One observation of particular interest concerns the asynchrony of second division in this cell line. If a cell is observed to undergo mitosis, we have observed that there are wide variations in the time of mitosis of the two daughter cells, i.e. the interval between generation and division may vary by 30 percent or more. This has held in all cases which we have observed to date.

Another study involving P388D macrophages shows them to be a useful subject for an asbestos-macrophage model system. At the same time, our observations with rectified objectives shows up particles of asbestos which are otherwise not discernible with ordinary optics. The system is therefore of interest since living macrophages recovering from

asbestos toxicity, as well as bearing asbestos fibers, can be observed for periods as long as months. This work has been performed in collaboration with Dr. Robert Tucker (C:LB) and Dr. Arthur Frank (Mt. Sinai Medical School), and has been submitted for publication.

At this writing, Tritiated Thymidine studies of the P388D cell cycle are in progress employing the Grain Counter.

B. The Real Time Picture Processor: This consists of 1) an Axiomat microscope including facilities for fluorescence, polarized light, differential interference measurements, as well as programmable stage focus and zoom motions; 2) a programmable illumination subsystem with provision for phase randomized laser illumination; 3) a programmable precision galvanometer-mirror scanner; 4) Plumbicon scanner; 5) a visible/infra-red rapid scan spectrometer; 6) a Quantimet 720 special purpose image analyzer; 7) Dicomed Display; 8) a custom console which includes a sonic digitizing tablet; 9) a buffer memory system with associated ultra-high speed A/D and D/A conversion systems for real time acquisition/display/storage of images from any system source; 10) the General Picture Processor; and, 11) a PDP8/e computer which controls all of the preceding elements.

During the year, construction of item 9, the buffer memory system, was completed. At this writing the subsystem is operational and is being debugged at Carman Engineering, Corvallis, Oregon. It will be installed and operational at the Image Processing Unit prior to the end of the fiscal year, exactly as scheduled. Items 1, 3, 4, 6, 7 and 8 are already fully constructed and interfaced to the PDP8/e. Thus, with the completion of the buffer memory system, work on living cell systems may begin as planned for FY 1977. Quantitative polarized light and differential interference measurements must await completion of the safety interlocks for the four-watt Argon-ion laser which are presently under construction, and should be completed during FY 1977.

Design and construction of the General Picture Processor, the high speed computing component of the RTPP, will begin (according to published specifications) during the Transitional Quarter and should be completed by the end of FY 1979.

Because of the modular nature of the RTPP, it is possible to use many of its components either stand-alone or as existing control connections. It is this modularity that will permit the beginning of living cell work and the continued pursuit of fixed cell analyses on the system during FY 1977.

C. Software: During the reporting period there was extensive software development for the RTPP as well as for the controlling PDP-10 system. The following are highlights of these tools:

1. PROCES: A set of image acquisition, processing and display programs for the PDP8/e involving the Axiomat its mirror scanner, and the Dicomed Display. Sequences of image transforms may be displayed. Only local hardware is required.

2. SOP: A set of image analysis programs coupling the Axiomat, the Plumbicon/Vidicon scanners and the Quantimet under control of the PDP8/e. Data from the so-called function computers may be acquired for each detected object. The program puts the Quantimet under partial PDP8/e control.

3. PROC10: This is a most advanced image processing software system running on the PDP-10. It allows the user to perform a very large number of transforms on RTPP acquired images, and display the results on devices such as the Tektronix 4012 Storage Tube. This complex represents a fusion of algorithms developed in our, as well as in other, laboratories over the years. Extensive facilities for handling boundaries, masks and windows, as well as raw and transformed images, make it particularly useful for direct biologist use.

4. MAINSAIL: In cooperation with the Stanford Artificial Intelligence in Medicine - SUMEX project, a PDP-10 based compiler for the RTPP was collaboratively specified and work on its implementation begun (by Clark Wilcox of SUMEX). This work is being sponsored by the NIH Biotechnology Resources Program Branch, DRR, as part of their major effort to make SAIL more generally available. MAINSAIL (in the GPP) should be available long before the hardware is completed.

5. GPPASM: MAINSAIL code for execution on a PDP-8 must be assembled. Work on such an assembler was begun during the reporting period.

6. PRDL: The"Procedural Description Language" which is the mechanism whereby the biologist will use the overall system, has been actively pursued during the year. The parser generator component of this language has been debugged and has been employed to check grammars for several other system components. (It has also been exported to the University of Rochester for course work in artificial intelligence). In operation, PRDL will call procedures now in PROC10 which in turn will be computed on the RTPP.

7. DDTG: This is the debugger/monitor for the entire Real Time Picture Processor. In addition to providing a mode for PDP-10 control of our local instrumentation, DDTG allows the apparatus to be used in an integrated way in stand-alone mode. All of the components (even those still to be constructed) of the RTPP are provided for in the DDTG software. In general, it provides for control of the microscope, the scanners, modification of light source, the Quantimet and its components, and the Dicomed. DDTG also services the manual controls of the system.

   DDTG was completed during the reporting period and is presently used for image acquisition and display on a daily basis. Doubtless, some bugs will be discovered when the (existing) software for the buffer memories is exercised, but this is a normal expectation and should offer no problems.

D. Algorithms:

1. Shape Descriptor: In addition to system software, an extremely significant development has been the creation of a new biological shape descriptor. This, in contrast to the various orthogonal transforms, allows both biologist and machine to refer explicitly to local regions of a boundary,to alter shape locally, and to express shape with variable precision depending upon such factors as resolution or magnification. This shape descriptor is of sufficient generality to apply to biologic shapes in general and not necessarily to microscopic objects alone. The ability to connote or denote local shape variations make it particularly felicitous for the description of changes in shape such as occur during embryogenesis, amoeboid motion, mitosis, etc. The generalization to three-dimensional objects, i.e. shapes of surfaces, seems feasible and will be pursued during the next fiscal year.

2. Optical Serial Sections: One of the fundamental operations of quantitative microscopy is accounting for out-of-focus information. If the thickness of a microscopic object exceeds the depth of focus of the objective, the microscopic image is involved with densities from out-of-focus planes. A first step in this problem was accomplished during the reporting period by adoption of one of the Algebraic reconstruction algorithms to remove out-of-focus information. This was shown to be effective in improving the image of a nucleolus present in a 35 micron thick section of nervous tissue. The immediate consequence of this development is the prospect of precision microdensitometry

in thick sections, limited only by considerations of spherical aberration. In a thick section, e.g. up to 250 microns or more in thickness, errors due to partial sectioning of objects are not significant while three-dimensional mapping results are possible without error prone reconstructions.

The computations involved are massive, but of a type which are relatively easily transferred tc parallel processors (e.g. optical computing devices) so that computing speeds eventually should not constitute an obstacle to adoption of such technics.

E. Miscellaneous Software and User Packages: Several components have been built to meet both special and more general long-term needs. These include a program to allow measurement of cell orientation in photographs of tissue cultures using the sonic digitizer and a reconstructed magnetic tape utility package for both image and alpha-numeric data.

High Resolution Computerized Tomography (Project No. Z01 CBD 08477-01 IMP):

Theoretically it should be possible to improve volume resolution in X-ray tomography by a factor of 10,000. If this is the case, it should be possible to detect tumors of the order of 1mm in diameter, i.e. at a stage very much prior to their phase of extensive and invasive growth. In order to test this assertion, we are preparing to reconstruct high resolution X-ray film images of thin slices (millimeters thick) of breast using weighted ART algorithms. A strategy to reduce the amount of computer time involving run length encoding of the X-ray images has been developed and is awaiting trial on real material. The combination of a favorable result with an effective strategy to reduce the computational load would warrant consideration of specifications for a new high resolution scanner.

Studies in Morphogenesis (Project No. Z01 CBD 08478-01 IMP):

A computer simulation of the forces to produce the early morphology of the neural plate in the Newt was completed. The accomplishment is significant in that the morphogenesis of the Newt neural plate is satisfactorily expressible in terms of the behavior of component cells. It is hoped to extend the time to the neural tube stage, but this will require the computer facilities of the RTPP.

Work on the patterns of pigmentation in the Zebra fish - a fundamental study of pigment cell migration from the neural crest - was begun during this year. This study will also require the powerful image acquisition/computer facilities of the RTPP.

Automated Grain Counting/Densitometer (Project No. Z01 CBD 08475-04 IMP:

In August 1975, we were officially informed by the new Director of the Division for Cancer Treatment, that his Division could not devote the requisite funds for construction of the four field model Grain Counters. His decision took cognizance of the successful accomplishment of the assigned mission, but he indicated that other equipment priorities were higher and more demanding.

It is our intention to:

1. Continue to employ the Grain Counter as a cytologic tool. To this end, we expect that Dr. Markovic, our Fogarty Fellow, will be occupied in part with the complete validation of the equipment.

2. We have in progress a three-volume description of the hardware which should be available to any interested group via NTIS. During the next year the software will be made similarly available.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08475-04 IMP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Automated Grain Counting/Densitometer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. E. Lipkin	Head, Image Process. Unit	IMP C
OTHER:	N. Markovic	Fogarty Fellow	IMP C
	M. Schultz	Electrical Engineer	IMP C
	G. Grosfeld	Computer Programmer	IMP C
	M. Wade	Biologist	IMP C

COOPERATING UNITS (if any)

None

LAB/BRANCH

Diagnosis Branch, Division of Cancer Biology and Diagnosis

SECTION

Image Processing Unit

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

SUMMARY OF WORK (200 words or less - underline keywords)

The fiscal decision to defer the construction of the four proposed field models has suspended this as a research project. The instrument continues to be used in cell kinetics measurements, autoradiography and image analysis of objects of interest in quantitative cytology.

The validation of the instrument on bone marrow and tissue culture material is proceeding as is the detailed documentation.

PHS-6040
(12-75)

In August 1975, we were officially informed by the new Director of the Division for Cancer Treatment that his Division could not devote the requisite funds for construction and maintenance of the promised four field model Grain Counters. His decision took cognizance of the successful accomplishment of the assigned mission, but he indicated that other equipment priorities were higher and more demanding.

We have in operation the prototype Grain Counter which is in regular use as applied to kinetic and cytomorphologic data particularly on P388D1 macrophages. We plan to continue to use this instrument for its original intended purpose. We also plan to continue its validation. To this end, the Fogarty Fellow, Dr. Markovic (who we expect to join us during the month of April), will devote a significant portion of his time. We expect that both bone marrow and tissue culture material will be used.

We have in progress a three-volume description of the hardware which, taken together with the off-the-shelf components, constitutes the Grain Counter. This detailed description will supplement published accounts, and will be available via the National Technical Information Service.

Prior to discontinuance of the field model phase, four sets of circuit boards for the field models were prepared. We are holding these as the property of DCT but with their approval would make them available to a competent individual desiring to duplicate the Grain Counter with grant or other funding.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CBD 08476-04 IMP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Automatic Cell Analysis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. E. Lipkin	Head, Image Process. Unit	IMP C
OTHER:	P. F. Lemkin	Computer Specialist	IMP C
	B. A. Shapiro	Expert Consultant	IMP C
	M. Schultz	Electrical Engineer	IMP C
	R. Gordon	Expert Consultant	IMP C
	M. Wade	Biologist	IMP C
	G. Grosfeld	Computer Programmer	IMP C
	R. Tucker	Fellow	LB C
	B. S. Lipkin	Computer Scientist	SRB RG

COOPERATING UNITS (if any)

G. Carman	Carman Electronics	Corvallis, Ore.
A. Frank	Mt. Sinai School of Medicine	New York, N.Y.
C. Wilcox	SUMEX-Stanford University	Stanford, Cal.

LAB/BRANCH

Diagnosis Branch, Division of Cancer Biology and Diagnosis

SECTION

Image Processing Unit

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	5.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Progress in constructing a hardware and software system for real time image analysis. It has reached a stage where it is possible to measure various surface markers in terms of cell cycle (kinetics) parameters (nuclear and cytoplasmic) of large, monolayer cultured cells such as P388D1 macrophages. In addition, progress on three-dimensional image reconstruction, shape descriptors, and image segmentation has been considerable. This is represented by development of a new biologically oriented shape descriptor, development of a strategy for scene segmentation in bone marrow, and algorithms for the improvement of optical serial sections and possible microdensitometry in thick slabs of tissue (up to .25mm).

A. Tissue Culture: Work in this area is to investigate biological and physical properties of cell lines which are of interest for automatic living cell tracking and analysis. For this purpose we have undertaken extensive time-lapse cinematographic studies, in particular on macrophages of line P388D1. This malignant cell line is characterized by many normal macrophage-like properties including phagocytosis of polystyrene beads, non-specific esterase activity, a surface Fc receptor, and no detectable theta antigen or surface immunoglobulin. In addition to these established (Koren et al) properties, we have observed a characteristic macrophage property, the formation of fusion multinucleated cells in culture and in the absence of a foreign body.

From the point of view of the cell cycle, there is evidence of occasional (we cannot yet estimate the frequency) wide variations in the time of mitosis of two daughter cells, i.e. the interval between generation and division may vary by thirty percent or more. This has held in all cases which we have observed to date.

Another study involving phagocytosis by P388D1 cells involved varying asbestos samples. Examples (standard asbestos preparations) of both crysotile and amosite asbestos were observed intracellularly in cultures maintained for months. An additional interesting feature is the apparent ability of the rectified optics employed to show the presence of asbestos fibers which would not be visible in ordinary microscopy. This work on what seems like a promising model system for asbestos macrophage interaction has been performed in collaboration with Dr. Robert Tucker (C:LB) and Dr. Arthur Frank (Mt. Sinai Medical School). A description of the system has been submitted to Nature for publication.

At this writing, tritiated thymidine studies of P388D1 cell line are in progress. The Grain Counter enables us to count grains and simultaneously measure nuclear size on several hundred individual cells for each selected time interval.

B. The Real Time Picture Processor: This is the local image acquisition/analysis facility which if necessary can operate in stand-alone manner under direct biologist control. It is a complex of the following items:

1. An Axiomat microscope including facilities for fluorescence, polarized light, differential interference measurements, as well as programmable stage, focus and zoom motions.

2. A programmable illumination subsystem with provision for phase randomized laser illumination. The controllable elements in the illumination subsystem include a continuously variable gray scale attenuator (circular wedge) and a continuously variable wavelength (interference wedge) both driven by high speed stepping motors.

3. A programmable precision galvanometer-mirror scanner which may be used for image acquisition as well as a microdensitometer. This device is diffraction limited.

4. A Plumbicon Scanner - with less precision, but about 600 times faster than the mirror scanner. It is an alternate digitizing device.

5. A visible through near infra-red rapid scan spectrometer. This will output 400 regularly spaced samples of a selected 300 nanometer-wide spectral range in about 20 milliseconds. The output is directly interfaceable to a computer and forms a basis for a third independent densitometric measuring system for the RTPP, as well as being indespensible in fluorescence studies,

6. A Quantimet 720 special purpose so-called "image analyzer." This can do simple processing on the Plumbicon images.

7. A high precision Dicomed Display (1024x1024 points, each of which is represented by 64 possible gray levels).

8. A custom console which includes a sonic digitizing tablet. These manual controls allow a biologist to point to objects and otherwise enter control information to the system while observing the microscope equivalent image on the QMT Display.

9. A buffer memory system with associated high speed A/D and D/A conversion systems for real time acquisition and/or display and/or storage of images from any system source.

10. The General Picture Processor - the high speed three-address computer specially designed for picture operations.

11. A PDP8/e Computer which controls all of the above elements at the instance of the biologist when in stand-alone operation.

During the year, construction of the buffer memory system, item 9, was completed. At this writing, the constituent 34 controller and memory cards are undergoing computer controlled testing at Carman Electronics in Corvallis, Oregon. As for the memory itself, the 2048 constituent integrated circuits are being tested for reliability both at the bit and pattern level. The programming for this testing was done by Carman Electronics and of itself constitutes a notable addition to the state-of-the-art. In addition, these programs will be invaluable for subsequent maintenance of the buffer memories.

The completed, tested, and debugged memory modules are scheduled for installation in the RTPP before the end of the reporting period - meeting our projections exactly.

Items 1, 3, 4, 6, 7 and 8 are already fully constructed and interfaced to the PDP8/e. Thus, with completion of the buffer memory system, work on living cell systems may begin as planned for in FY 1977. Quantitative polarized light and differential interference measurements must await completion of the safety interlocks for the Argon-ion laser presently under construction. This is because these extremely useful measures require at least two orders of magnitude greater illumination light flux than absorption densitometry. The safety features are an absolute necessity and it is the policy of the laboratory that the laser may not be fired-up until the system is completed and tested with a 20 milliwatt helium neon laser.

During the next fiscal year, it is planned to devote our major hardware effort to completing the design and beginning the construction of the high speed computing component of the RTPP (the General Picture Processor - GPP). Preliminary design requirements have been published as have functional specifications. A very conservative estimate projects the completion (including documentation which alone is six man months of senior engineering time) of the GPP by the end of FY 1979. Certainly portions of this subsystem will be productively operational long before then, i.e. prior to the end of FY 1978.

Because of the modular nature of the RTPP, it is possible to use many of its components either stand-alone or as existing semiautomatic subsystems. The system effectively is reconstructed or reconfigured by changing software. It is this modularity that will permit the beginning of living cell work and the continued pursuit of fixed cell analyses on the system during FY 1977.

C. Software: During the reporting period there was extensive software development for the RTPP as well as for the controlling PDP-10 system. In some cases the software packages are "intermediate." That is, they are logical groupings of functions which for design and testing purposes are brought together, but which eventually may be reseparated since some functions may more efficiently reside on different processors.

The following are highlights of software developments:

1. PROCES: This system, constructed by Lemkin and Shapiro is an articulated group of image processing programs which may be run on-line by nonprogrammers. A variety of image processing/analysis features are available. These include scaling, thresholding, slicing, the option of displaying and/or disk output of image transforms, histograms, gradients, Laplacians, etc. Operations involving two images with a third as output include pointwise addition, subtraction, multiplication, division, maximum and minimum. In addition, there is a boundary generating routine. All operations are performed on standard (for us) 256x256 images with 256 gray level representation. PROCES was written in standard PDP8/e Fortran II-Sabr and operates under the OS/8 stand-alone monitor. It is learned within an hour or so and can put up on the display a standard size image in about one minute when it is resident on a disk file. The software allows up to 16 standard images to be placed on the Dicomed at any one time.

   This sort of software was in part written to allow biologists to see what some of the real needs of the larger DDTG system would really be as opposed to the usual speculation involved in constructing large software systems.

   PROCES is exportable in the sense that it will run under OS/8 on any PDP8/e with 32K of core, an extended arithmetic element and random access storage. Output may be on a hard copy device such as a DECwriter if no display is available.

2. SOP: Just as in PROCES, the mirror scanner, Dicomed Display, and PDP8/e are brought together as an operating unit, so in SOP are the Quantimet 720 and PDP8/e brought together for programmed data acquisition and analysis. Also, similar to PROCES, SOP makes extensive use of existing system software. The set of programs was built

as an intermediate software package by Peter Lemkin and has functioned well as a stand-alone. In particular, it allows the nonprogrammer to dynamically reconfigure the Quantimet so that no manual front panel operations are necessary. For example, one can set detector thresholds, select various functions of different detector or analyzer components, move the live frame (detection mask)throughout the field and print out data on command.

Provision for the expected buffer memories is also available as is Dicomed display of Quantimet images which will be physically possible with the advent of the buffer memories.

3. PROC10: This is a most advanced image processing software system constructed by Lemkin, Gordon and Shapiro running on the PDP-10. Images which have been prepared or processed on this PDP-8 or compatibly formatted to our 256x256x8 bit standard may be transported to the PDP-10 by a variety of modes. PROC10 includes all of the operations in PROCES as well as an additional operation on pictures (more than 40) and boundary and window operations as well. A whole series of procedures which operate on linear structures such as lines, curves, boundaries, etc. are available. In addition, there are a very rich range of orthogonal transforms (Fourier, Karhunen, Loeve, etc. and their inverses).

An important features of PROC10 is the fact that under the PDP-10 monitor it supports image displays on the GT40 and various Tektronix storage tube terminals, i.e. the 4012. Even a standard soft display such as the Tektronix 4023 may be used under PROC10 for image analysis. Within the I/O package are Gordon's halftone routines which were designed for storage tube work on X-ray scanner images.

The logical and control features of PROC10 will eventually be brought under the Procedural Description Language, while its "number crunching" burden will be shifted to the GPP as it is brought up - a much more economical as well as much faster computer.

4. MAINSAIL: A compiler for the GPP is being written by Clark Wilcox of SUMEX as part of an experiment in artificial intelligence software. MAINSAIL is in one sense an improvement over the SAIL language (Stanford Artificial Intelligence Language) and its use in the GPP will offer no serious problems from the user viewpoint. It is planned that NIH will be one of the initial test sites for MAINSAIL both for the PDP-10 and for our special purpose picture processor. It is proposed that SUMEX

produce compilers for the PDP-10,runtime routines for the PDP-10 and a compiler for our GPP. They expect that they will complete the compiler long before the physical machine is available. This is particularly desirable since it will be necessary for full hardware debugging.

5. GPPASM: The output of MAINSAIL will be statements in assembly language. An assembler to handle this output should reside on the PDP-8. It must be very simple and very fast in order to run at all satisfactorily on such a small machine. A beginning has been made on construction of such an assembler by Gerson Grosfeld. The schedule calls for completion of the assembler soon after Wilcox completes the MAINSAIL compiler.

6. Procedural Description Language - PRDL: PRDL will be used to model biologic cell images. Our use, or rather concept, of a model is somewhat different than that usually encountered. To us, a model is an easily modifiable logical structure whose raison d'etre is that it serves as part of a control structure directing complex operations such as scene analysis. When we use the term model, it is in no sense an object in itself.

PRDL uses some of the concepts employed by other procedural description languages such as PLANNER, CONNIVER and new SAIL. PRDL is being implemented in new SAIL as an interpreter. It represents an extensive reworking of MLAB, an interactive modeling computer system running on the PDP-10. The extension of MLAB to PRDL involves the addition of new data types and operators to manipulate them.

In developing PRDL, it is necessary that considerable flexibility exist so that rapid and clean changes in the language can be made. For this purpose, a parser generator is not only convenient but a necessity, especially in the context of development of a language by a small group. This is particularly true as work with the system demonstrates the necessity for new constructs.

Accordingly, PRDL is designed so that its front-end parser is generated by supplying a BNF grammar specification to a Parser Generator. The resultant parser then replaces the current parser that exists in MLAB. The latter consists of hand-coded tables and is therefore very difficult to modify and expand.

In addition to its primary use, the Parser Generator has been used to create a parser for the GPP compiler which will run on the PDP-10. It is possible to modify the Parser Generator so that it produces a parser for other languages on other machines, such as standard Fortran or, for example, a PDP-11.

Interest in the Parser Generator beyond the confines of NIH has already resulted in its use at the University of Rochester in the artificial intelligence program there.

7. DDTG: DDTG, the creation of Peter Lemkin, is constructed for user and/or computer control of the Real Time Picture Processor (RTPP). Functionally, it is more than a simple combination of monitor and debugging facilities. As the RTPP operating system, it is required to interpret direct user commands, or a string of commands generated by user-PDP-10 interaction. In addition, it provides access and control (at the machine language level) of major memory structures (PDP8/e core, GPP program memory, general register memory, and buffer memories). It also provides full control for a variety of image acquisition and low level analytic peripherals. Further, DDTG has the ability to store, retrieve and execute PDP8/e and/or GPP programs. Since when the RTPP is in stand-alone mode, DDTG is used as much by biologists as by computer scientists, the user interface allows many high level and seeming English command constructs.

DDTG was completed during this reporting period and has been debugged with respect to RTPP hardware presently in operation. A normal expectation is that when the buffer memories are installed, a few additional bugs will be revealed, but on the basis of previous experience, this should offer no serious problems.

D. Algorithms:

1. Shape Descriptor: In addition to system software, an extremely significant development has been the creation of a new biological Shape Descriptor. This, in contrast to the various orthogonal transforms, allows both biologist and machine to refer explicitly to local regions of a boundary, to alter shape locally, and to express shape with variable precision depending upon such factors as resolution or magnification. This Shape Descriptor is of sufficient generality to apply to biologic shapes in general and not necessarily to microscopic objects alone.

The ability to connote or denote local shape variations make it particularly felicitous for the description of changes in shape such as occur during embryogenesis, amoeboid motion, mitosis, etc. The generalization to three-dimensional objects, i.e. shapes of surfaces, seems feasible and will be pursued during the next fiscal year.

The Descriptor itself consists of a series of parameters (signed curvature, angular deviation and arc length), the length of the list in part depending on the complexity of the boundary. It is position and rotation independent and is therefore a close approximation to the ideal Shape Descriptor from the biologist's viewpoint. The list parameters lends itself to prediction of shape directly, a property which is not true of the orthogonal transforms.

We term the generator of the Descriptor the "circle transform" and it and its inverse are part of the procedures available in PROC10.

2. Optical Serial Sections: When a section of material is observed by light microscopy, one does not simply see an image of the focal plane. The image is always somewhat obscured by superimposed blurred images of the structures above and below the focal plane. This effect is lessened by Nomarski optics, but is still present. We have developed a computational method which actually removes the out-of-focus images. The result is a sharper image of the focal plane itself.

Our computational algorithm is mathematically the same as the one developed by Richard Gordon for computerized tomography for reconstruction from projections. It is one of the ART algorithms, namely constrained, weighted ART3.

The reconstructed image should be as superior to the direct image as the computerized tomogram is to the classical tomogram. Because computerized thin sectioning requires fewer mechanical sections with their loss of material and distortions, it may be the method of choice for quantitative three-dimensional investigations. It is certainly the method of choice when studying living specimens.

An image is recorded by the galvanometer scanner attached to the Axiomat microscope (part of the Real Time Picture Processor, or RTPP). At present this image is recorded on digital tape and carried to the DCRT PDP-10 computer. The

program which isolates the focal image from the recorded image is called ARTHIN and is written in SAIL. It makes use of components of the picture processing package PROC10 developed by the Image Processing Unit. Its input and output are fully compatible with PROC10. Moreover, ARTHIN is written in such a way as to be implementable on the RTPP when the buffer memories are operational and MAINSAIL is available.

The program has been successfully applied to the nucleolus of a mouse brain cell in a 35 micron section stained with Gallocyanin. The focal image was isolated in a slab of approximate thickness of 0.164 microns. (Each picture element represented an approximate cube 0.164 microns on a slide.)

The future course of this work will include:

a) Low resolution reconstruction of a three-dimensional object of known structure prepared from pieces of thin coverslips surrounded by stained immersion oil.

b) High resolution reconstruction of single cells in a through focal series, followed by mechanical thin sectioning for comparison.

c) Reprogramming on the RTPP for increased computing speed and for making the images rapidly available to a user of the RTPP.

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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CBD 08477-01 IMP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

High Resolution Computerized Tomography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Gordon	Expert Consultant	IMP C

COOPERATING UNITS (if any)

L. Silver	G. W. University	Washington, D.C.
D. S. Rigel	G. W. University	Washington, D.C.

LAB/BRANCH

Diagnosis Branch, Division of Cancer Biology and Diagnosis

SECTION

Image Processing Unit

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	0

SUMMARY OF WORK (200 words or less - underline keywords)

We are attempting to improve computerized tomography by a new approach which permits maximal use of the spatial resolution attainable with X-rays. Tests are being carried out on thinly sliced breast tissue. The results are expected to lead to the design of X-ray diagnostic equipment with potential for early tumor detection.

PHS-6040
(12-75)

The major limitation in current instruments for computerized tomography is the wide sampling aperture of their X-ray collimators. X-ray film readily records details as small as 0.1mm, whereas the volume elements reconstructed by X-ray scanners typically have dimensions of 1mm x 1mm x 10mm. It is our aim to reconstruct volume elements 0.1mm on each side, or 10,000 times finer than current instruments. This would make possible the detection of tumors of millimeter or less dimensions. Such tumors are generally not yet vascularized and are undergoing a long period of slow growth. If breast tumors could be detected at this early stage and removed, more women could be expected to survive the disease.

The primary difficulty in attempting finer resolution is the large amount of computer time involved. Our approach to this problem has been to reduce the data to manageable proportions by using run length encoding of the X-ray readings, which preserves most of the recorded information. When projected back across the section of tissue being reconstructed, these run lengths divide the section into a number of polygons of various sizes. Using the areas of the polygons as their weights, we may reconstruct the section with any weighted ART algorithm. The set of computer programs for carrying out these operations is nearly complete. They will be tested on X-ray contact prints of thin slices of fresh human breast tissue obtained by permission from autopsies or mastectomies. (Slicing is carried out in a cold room using a meat slicer.)

If reconstructions of the X-ray contact prints are satisfactory, recommendations based on these findings could be made for construction of a high resolution breast scanner.

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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CBD 08478-01 IMP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies in Morphogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. Gordon Expert Consultant IMP C

COOPERATING UNITS (if any)
A. G. Jacobson University of Texas Austin, Texas

LAB/BRANCH
Diagnosis Branch, Division of Cancer Biology and Diagnosis

SECTION
Image Processing Unit

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

SUMMARY OF WORK (200 words or less - underline keywords)

We have identified the forces that are necessary and sufficient to produce the keyhole shape of the Newt embryo neural plate from a hemispheric sheet of cells one cell thick. These forces are: 1) a regionally programmed shrinkage of the surface of the neural plate (accomplished by contraction of the apical surfaces of the neural plate cells and elongation of these cells perpendicular to the plate); 2) displacement of the whole sheet caused by elongation of the notochord or the overlying neural plate in the anterior-posterior direction.

Since each cell in the neural plate contributes to the motion of the whole sheet, we used a computer simulation and mathematical analysis ('morphodynamics') to deduce the morphogenesis of the neural plate from the behavior of its component cells.

Preparation of the manuscript describing this five year study has been completed. We expect to expand this investigation in the following directions:

1. The development of the eye also seems to follow a regionally programmed shrinkage pattern. Since it is approximately radially symmetric, an effectively one-dimensional computer simulation has been written. This program allows considerable shearing between the cells. It will be used to attempt a quantitative analysis of the shaping of the eye.

2. The study of the neural plate will be taken through neurulation to the stage of neural tube formation. This will require a three-dimensional simulation of a sheet of cells in space. Since the major driving force during neurulation seems to be elongation of the notochordal region, we will undertake three-dimensional reconstruction of the notochordal cells from serial sections. The cell shapes may give some clues to the mechanism of notochord elongation.

Publications:

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